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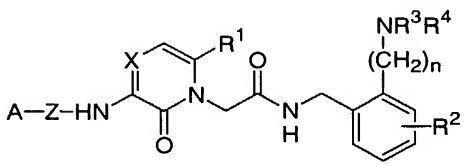
(54) Title: THROMBIN INHIBITORS



(I)

(57) Abstract: Compounds of the invention are useful in inhibiting thrombin and associated thrombotic occlusions having the following structure: or a pharmaceutically acceptable salt thereof, wherein R^3 and R^4 are independently selected from the group consisting ofhydrogen,-C(O)R^5, where R^5 is selected from the group consisting of $OC(CH_3)$, OCH_3, CH_3, $NHCH_3$, and OCH_2R^6, where R^6 is phenyl; C_{1-4}alkyl,C_{3-7}cycloalkyl,-$(CH_2)_{1-2}$ R^7, where R^7 is selected from the group consisting of C_{3-7}cycloalkyl and phenyl,-SO_2R^8, where R^8 is C_{1-4}alkyl.

TITLE OF THE INVENTION

THROMBIN INHIBITORS

BACKGROUND OF THE INVENTION

Thrombin is a serine protease present in blood plasma in the form of a precursor, prothrombin. Thrombin plays a central role in the mechanism of blood coagulation by converting the solution plasma protein, fibrinogen, into insoluble fibrin.

Edwards et al., J. Amer. Chem. Soc., (1992) vol. 114, pp. 1854-63, describes peptidyl a-ketobenzoxazoles which are reversible inhibitors of the serine proteases human leukocyte elastase and porcine pancreatic elastase. European Publication 363 284 describes analogs of peptidase substrates in which the nitrogen atom of the scissile amide group of the substrate peptide has been replaced by hydrogen or a substituted carbonyl moiety. Australian Publication 86245677 also describes peptidase inhibitors having an activated electrophilic ketone moiety such as fluoromethylene ketone or a-keto carboxyl derivatives. R. J. Brown et al., J. Med. Chem., Vol. 37, pages 1259-1261 (1994) describes orally active, non-peptidic inhibitors of human leukocyte elastase which contain trifluoromethylketone and pyridinone moieties. H. Mack et al., J. Enzyme Inhibition, Vol. 9, pages 73-86 (1995) describes rigid amidino-phenylalanine thrombin inhibitors which contain a pyridinone moiety as a central core structure.

SUMMARY OF THE INVENTION

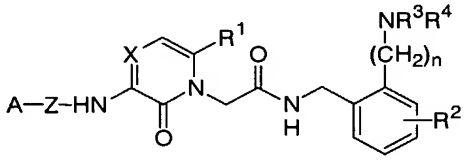
The invention includes compounds for inhibiting loss of blood platelets, inhibiting formation of blood platelet aggregates, inhibiting formation of fibrin, inhibiting thrombus formation, and inhibiting embolus formation in a mammal, comprising a compound of the invention in a pharmaceutically acceptable carrier. These compounds may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents. The compounds can be added to blood, blood products, or mammalian organs in order to effect the desired inhibitions.

The invention also includes a compound for preventing or treating unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, ocular build up of fibrin, and reocclusion or restenosis of recanalized vessels, in a mammal, comprising a compound of the invention in a pharmaceutically acceptable carrier. These compounds may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents.

The invention also includes a method for reducing the thrombogenicity of a surface in a mammal by attaching to the surface, either covalently or noncovalently, a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION AND
PREFERRED EMBODIMENTS

Compounds of the invention are useful as thrombin inhibitors and have therapeutic value in for example, preventing coronary artery disease. The invention includes compounds having the following structure:



and pharmaceutically acceptable salts thereof, wherein

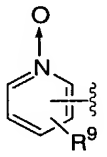
n is 1 or 2;

A is

1) a 6-membered non-heterocyclic unsaturated ring system, unsubstituted, monosubstituted, disubstituted, or trisubstituted, same or different, with C_{1-4} alkyl;

2) a 6-membered heterocyclic unsaturated or saturated ring system wherein 1 ring atom is selected from the group of heteroatoms consisting of N, O and S, wherein the ring carbons are unsubstituted, monosubstituted, disubstituted, or trisubstituted, same or different, with C_{1-4} alkyl, or

3)



, where R^9 is hydrogen or C_{1-8}alkyl;

Z is $-(CH_2)_{2-4}-$, $-CF_2(CH_2)_{1-3}-$, or $-(CH_2)_{1-3}SO_2-$;

X is CH or N;

R^1 is halogen;

R^2 is hydrogen or halogen; and

R^3 and R^4 are independently selected from the group consisting of

hydrogen,

$-C(O)R^5$, where R^5 is selected from the group consisting of $OC(CH_3)$, OCH_3, CH_3, $NHCH_3$, and OCH_2R^6, where R^6 is phenyl;

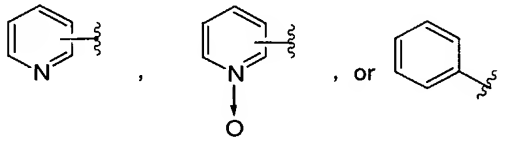
C_{1-4} alkyl,

C_{3-7} cycloalkyl,

$-(CH_2)_{1-2}R^7$, where R^7 is selected from the group consisting of C_{3-7} cycloalkyl and phenyl,

$-SO_2R^8$, where R^8 is C_{1-4}alkyl.

In a class of compounds and pharmaceutically acceptable salts thereof, R^1 is Cl, and A is



In a group of this class of compounds and pharmaceutically acceptable salts thereof, R^3 and R^4 are independently selected from the group consisting of

hydrogen,

$-C(O)R^5$, where R^5 is selected from the group consisting of $OC(CH_3)$, OCH_3, CH_3, $NHCH_3$, and OCH_2R^6, where R^6 is phenyl;

C_{1-4} alkyl,

cyclobutyl,

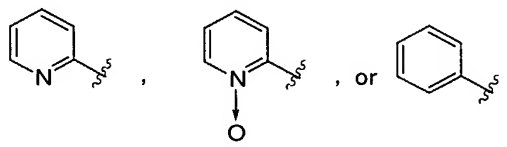
cyclopentyl,

$-(CH_2)_{1-2}R^7$, where R^7 is selected from the group consisting of cyclopropyl and phenyl, and

$-SO_2CH_3$.

In a subgroup of this group of compounds and pharmaceutically acceptable salts thereof, X is CH or N; Z is $-CH_2CH_2-$, $-CF_2CH_2-$, -CH2SO2-;

A is



;

R^1 is Cl;

R^2 is hydrogen or Cl;

R^3 and R^4 are independently selected from the group consisting of

hydrogen,

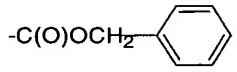
$-C(O)OC(CH_3)_3$,

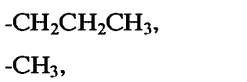
$-C(O)OCH_3$,

$-C(O)CH_3$

$-SO_2CH_3$,

$-C(O)NHCH_3$





$-CH_2CH_2CH_3$,

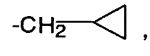
$-CH_3$,

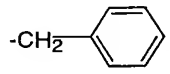
$-CH_2CH_3$,

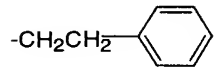
$-CH_2CH_2CH_2CH_3$,

$-CH_2CH(CH_3)_2$,

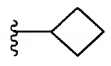
$-C(CH_3)_3$,

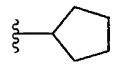






$-CH(CH_3)_2$,

 , and

 .

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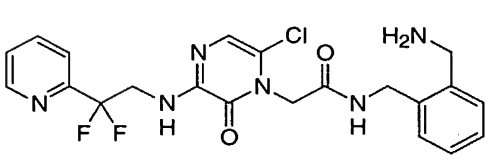
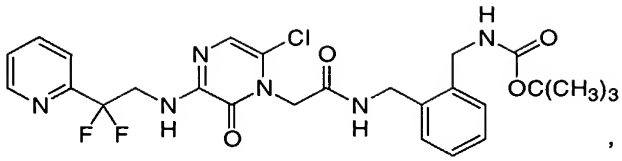
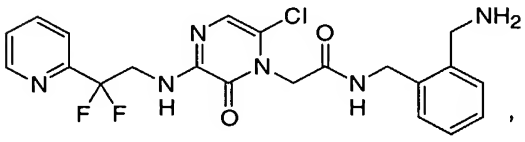
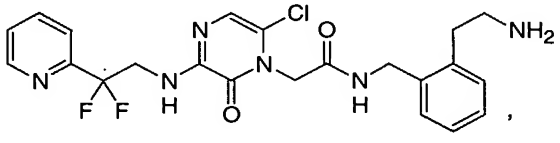
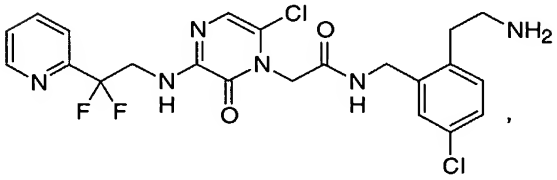
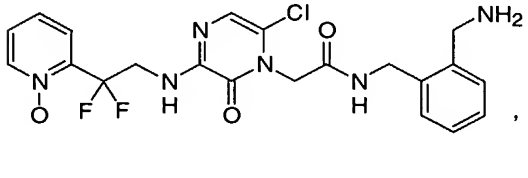
 ,

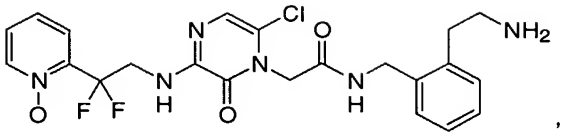
 ,

 ,

 ,

 ,

 ,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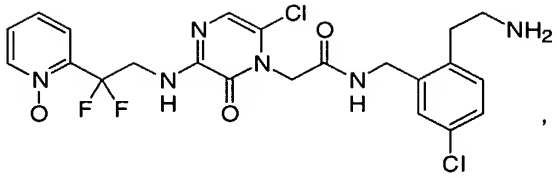
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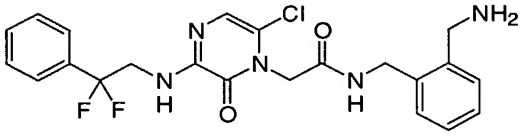
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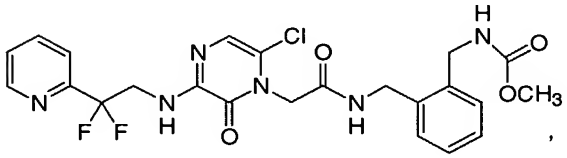
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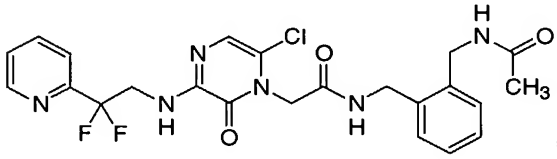
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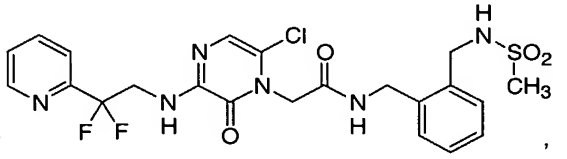
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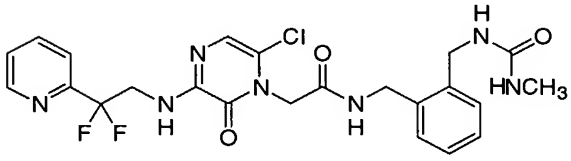
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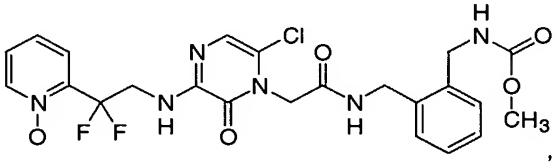
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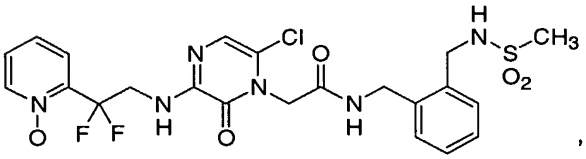
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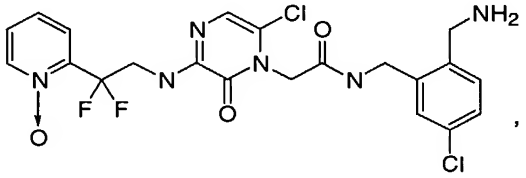
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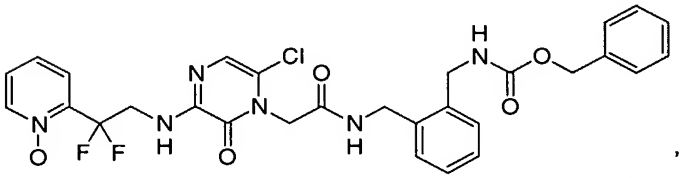
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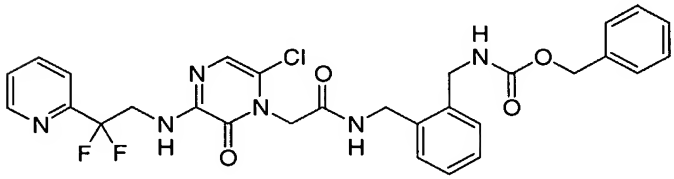
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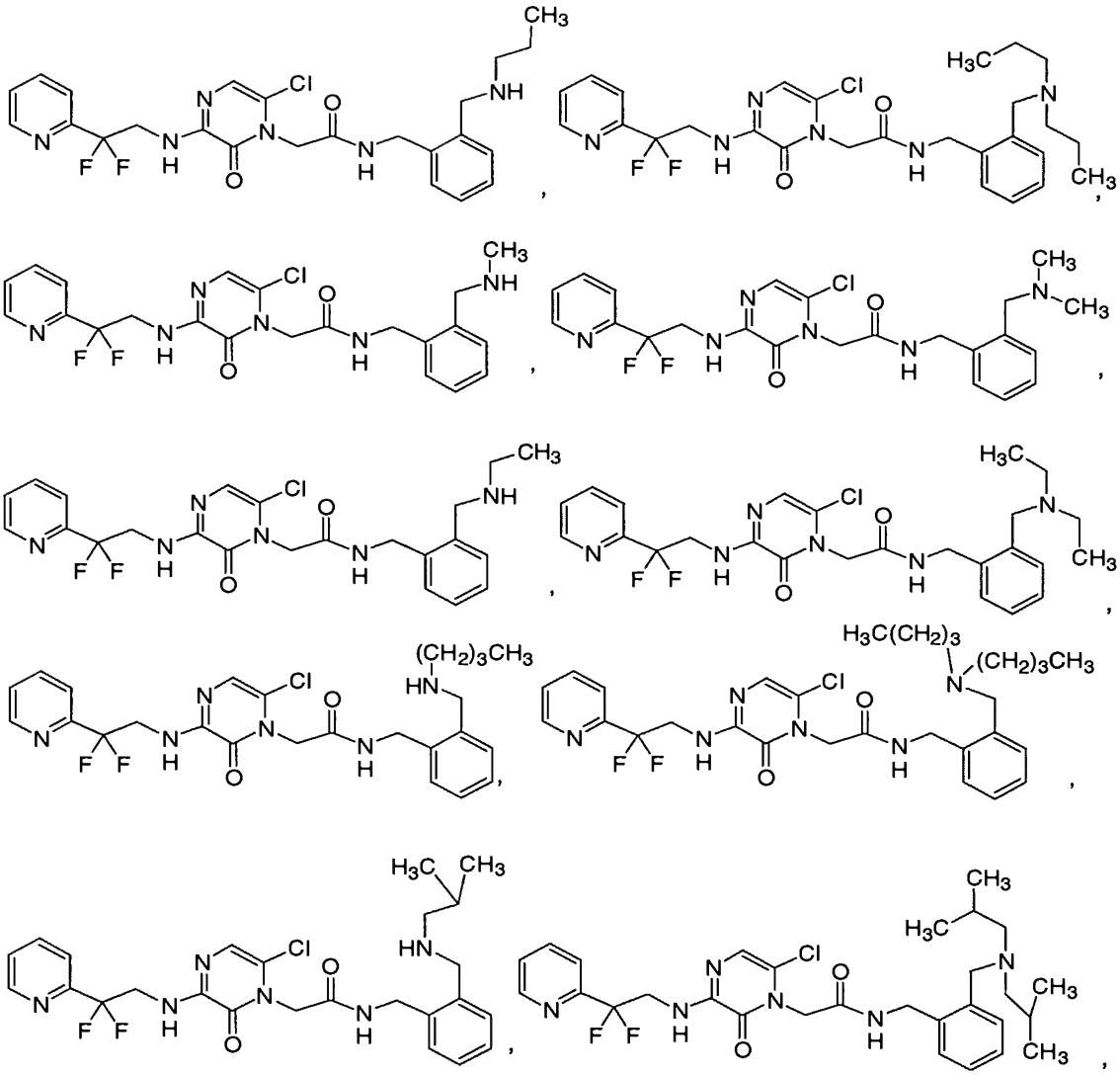
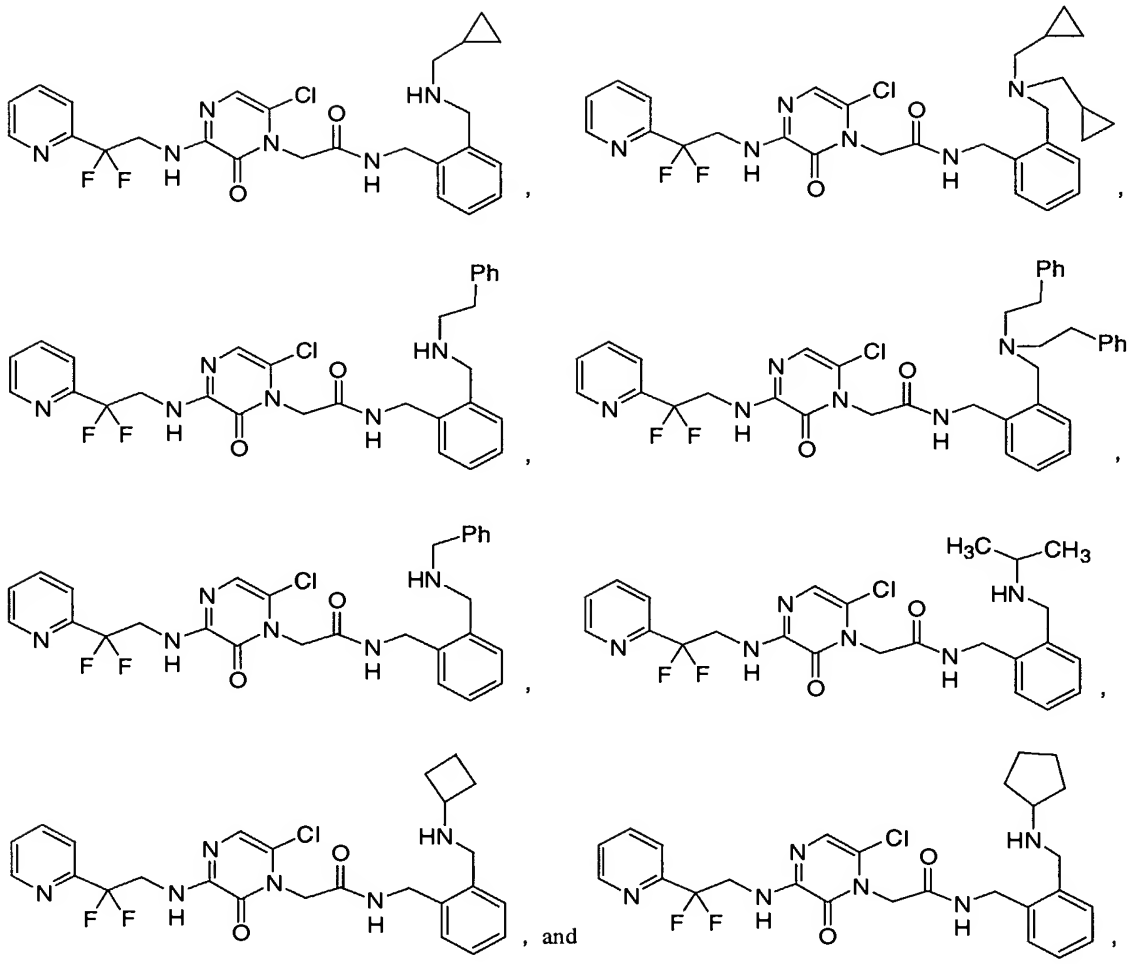




and pharmaceutically acceptable salts thereof.

The compounds of the present invention, may have chiral centers and occur as racemates, racemic mixtures and as individual diastereomers, or enantiomers with all isomeric forms being included in the present invention. The compounds of the present invention may also have polymorphic crystalline forms, with all polymorphic crystalline forms being included in the present invention.

When any variable occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

Some abbreviations that may appear in this application are as follows:

ABBREVIATIONS

Designation	
$(Boc)_2O$	di-t-butyl dicarbonate
DAST	diethylaminosulfurtrifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DMF	dimethylformamide
DPPA	diphenylphosphoryl azide
EtOH	ethanol
HCl	hydrochloric acid
HOAc	acetic acid
IPrOH	2-propanol
KOH	potassium hydroxide
LAH	lithium aluminum hydride
MCPBA	m-chloroperoxybenzoic acid
MeOH	methanol
$N_3PO(Ph)_2$	diphenyl phosphoryl azide
$NaBH_4$	sodium borohydride
NaN_3	sodium azide
NCS	N-chlorosuccinimide
$P(Ph)_3$	triphenyl phosphine
$Pd(PPh_3)_4$	tetrakis triphenylphosphine palladium
$PhCH_3$	toluene
$POBr_3$	phosphorous oxybromide
TEA	triethylamine
Tf_2O	trifluoromethane sulfonic anhydride
$Zn(CN)_2$	zinc cyanide

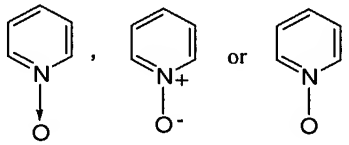
As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms (Me is methyl, Et is ethyl, Pr is propyl, Bu is butyl); "alkoxy" represents a linear or branched alkyl group of indicated number of carbon atoms attached through an oxygen bridge; "halogen", as used herein, means fluoro, chloro, bromo and iodo; and "counterion" is used to represent a small, single negatively-charged species, such as chloride, bromide, hydroxide, acetate, trifluoroacetate, perchlorate, nitrate, benzoate, maleate, sulfate, tartrate, hemitartrate, benzene sulfonate, and the like.

The term "cycloC3-7alkyl" is intended to include non-heterocyclic saturated rings such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl, and the like.

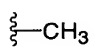
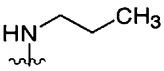
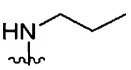
The term "aryl" as used herein except where noted, represents a stable 6- to 10-membered mono- or bicyclic non-heterocyclic unsaturated ring system such as phenyl, or naphthyl. The aryl ring can be unsubstituted or substituted with one or more of C_{1-4} lower alkyl; hydroxy; alkoxy; halogen; amino.

The term "heterocycle" or "heterocyclic ring", as used herein except where noted, represents a stable 5- to 7-membered mono- or bicyclic or stable 7- to 10-membered bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. Especially useful are rings containing one oxygen or sulfur, one to four nitrogen atoms, or one oxygen or sulfur combined with one or two nitrogen atoms. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic groups include piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazoyl, benzopyranyl, benzothiazolyl, benzoxazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, tetrazole, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl. Morpholino is the same as morpholinyl.

The pyridyl N-oxide portion of the compounds of the invention are structurally depicted using conventional representations



which have equivalent meanings.

In this specification methyl substituents may be represented by  or  . For example, the structures  and  have equivalent meanings.

The pharmaceutically-acceptable salts of the compounds of Formula I (in the form of water- or oil-soluble or dispersible products) include the conventional non-toxic salts such as those derived from inorganic acids, e.g. hydrochloric, hydrobromoic, sulfuric, sulfamic, phosphoric, nitric and the like, or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of acid addition salts include acetate, adipate, alginate, aspartate, benzoate,

benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, sulfate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others.

Thrombin Inhibitors - Therapeutic Uses- Method of Using

Anticoagulant therapy is indicated for the treatment and prevention of a variety of thrombotic conditions, particularly coronary artery and cerebrovascular disease. Those experienced in this field are readily aware of the circumstances requiring anticoagulant therapy. The term "patient" used herein is taken to mean mammals such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats, and mice.

Thrombin inhibition is useful not only in the anticoagulant therapy of individuals having thrombotic conditions, but is useful whenever inhibition of blood coagulation is required such as to prevent coagulation of stored whole blood and to prevent coagulation in other biological samples for testing or storage. Thus, the thrombin inhibitors can be added to or contacted with any medium containing or suspected of containing thrombin and in which it is desired that blood coagulation be inhibited, e.g., when contacting the mammal's blood with material selected from the group consisting of vascular grafts, stents, orthopedic prosthesis, cardiac prosthesis, and extracorporeal circulation systems.

Compounds of the invention are useful for treating or preventing venous thromboembolism (e.g. obstruction or occlusion of a vein by a detached thrombus; obstruction or occlusion of a lung artery by a detached thrombus), cardiogenic thromboembolism (e.g. obstruction or occlusion of the heart by a detached thrombus), arterial thrombosis (e.g. formation of a thrombus within an artery that may cause infarction of tissue supplied by the artery), atherosclerosis (e.g. arteriosclerosis characterized by irregularly distributed lipid deposits) in mammals, and for lowering the propensity of devices that come into contact with blood to clot blood.

Examples of venous thromboembolism which may be treated or prevented with compounds of the invention include obstruction of a vein, obstruction of a lung artery (pulmonary embolism), deep vein thrombosis, thrombosis associated with cancer and cancer chemotherapy,

thrombosis inherited with thrombophilic diseases such as Protein C deficiency, Protein S deficiency, antithrombin III deficiency, and Factor V Leiden, and thrombosis resulting from acquired thrombophilic disorders such as systemic lupus erythematosus (inflammatory connective tissue disease). Also with regard to venous thromboembolism, compounds of the invention are useful for maintaining patency of indwelling catheters.

Examples of cardiogenic thromboembolism which may be treated or prevented with compounds of the invention include thromboembolic stroke (detached thrombus causing neurological affliction related to impaired cerebral blood supply), cardiogenic thromboembolism associated with atrial fibrillation (rapid, irregular twitching of upper heart chamber muscular fibrils), cardiogenic thromboembolism associated with prosthetic heart valves such as mechanical heart valves, and cardiogenic thromboembolism associated with heart disease.

Examples of arterial thrombosis include unstable angina (severe constrictive pain in chest of coronary origin), myocardial infarction (heart muscle cell death resulting from insufficient blood supply), ischemic heart disease (local anemia due to obstruction (such as by arterial narrowing) of blood supply), reocclusion during or after percutaneous transluminal coronary angioplasty, restenosis after percutaneous transluminal coronary angioplasty, occlusion of coronary artery bypass grafts, and occlusive cerebrovascular disease. Also with regard to arterial thrombosis, compounds of the invention are useful for maintaining patency in arteriovenous cannulas.

Examples of atherosclerosis include arteriosclerosis.

Examples of devices that come into contact with blood include vascular grafts, stents, orthopedic prosthesis, cardiac prosthesis, and extracorporeal circulation systems

The thrombin inhibitors of the invention can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixers, tinctures, suspensions, syrups, and emulsions. Likewise, they may be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as an anti-aggregation agent. For treating ocular build up of fibrin, the compounds may be administered intraocularly or topically as well as orally or parenterally.

The thrombin inhibitors can be administered in the form of a depot injection or implant preparation which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber or other polymers manufactured by the Dow-Corning Corporation.

The thrombin inhibitors can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles.

Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

The thrombin inhibitors may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The thrombin inhibitors may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinlypyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxyethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the thrombin inhibitors may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

The dosage regimen utilizing the thrombin inhibitors is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Oral dosages of the thrombin inhibitors, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 30 mg/kg/day, preferably 0.025-7.5 mg/kg/day, more preferably 0.1-2.5 mg/kg/day, and most preferably 0.1-0.5 mg/kg/day (unless specificed otherwise, amounts of active ingredients are on free base basis). For example, an 80 kg patient would receive between about 0.8 mg/day and 2.4 g/day, preferably 2-600 mg/day, more preferably 8-200 mg/day, and most preferably 8-40 mg/kg/day. A suitably prepared medicament for once a day administration would thus contain between 0.8 mg and 2.4 g, preferably between 2 mg and 600 mg, more preferably between 8 mg and 200 mg, and most preferably 8 mg and 40 mg, e.g., 8 mg, 10 mg, 20 mg and 40 mg. Advantageously, the thrombin inhibitors may be administered in divided doses of two, three, or four times daily. For administration twice a day, a suitably prepared medicament would contain between 0.4 mg and 4 g, preferably between 1 mg and 300 mg, more preferably between 4 mg and 100 mg, and most preferably 4 mg and 20 mg, e.g., 4 mg, 5 mg, 10 mg and 20 mg.

Intravenously, the patient would receive the active ingredient in quantities sufficient to deliver between 0.025-7.5 mg/kg/day, preferably 0.1-2.5 mg/kg/day, and more preferably 0.1-0.5 mg/kg/day. Such quantities may be administered in a number of suitable ways, e.g. large volumes of low concentrations of active ingredient during one extended period of time or several times a day, low volumes of high concentrations of active ingredient during a short period of time, e.g. once a day. Typically, a conventional intravenous formulation may be prepared which contains a concentration of

active ingredient of between about 0.01-1.0 mg/ml, e.g. 0.1 mg/ml, 0.3 mg/ml, and 0.6 mg/ml, and administered in amounts per day of between 0.01 ml/kg patient weight and 10.0 ml/kg patient weight, e.g. 0.1 ml/kg, 0.2 ml/kg, 0.5 ml/kg. In one example, an 80 kg patient, receiving 8 ml twice a day of an intravenous formulation having a concentration of active ingredient of 0.5 mg/ml, receives 8 mg of active ingredient per day. Glucuronic acid, L-lactic acid, acetic acid, citric acid or any pharmaceutically acceptable acid/conjugate base with reasonable buffering capacity in the pH range acceptable for intravenous administration may be used as buffers. Consideration should be given to the solubility of the drug in choosing an The choice of appropriate buffer and pH of a formulation, depending on solubility of the drug to be administered, is readily made by a person having ordinary skill in the art.

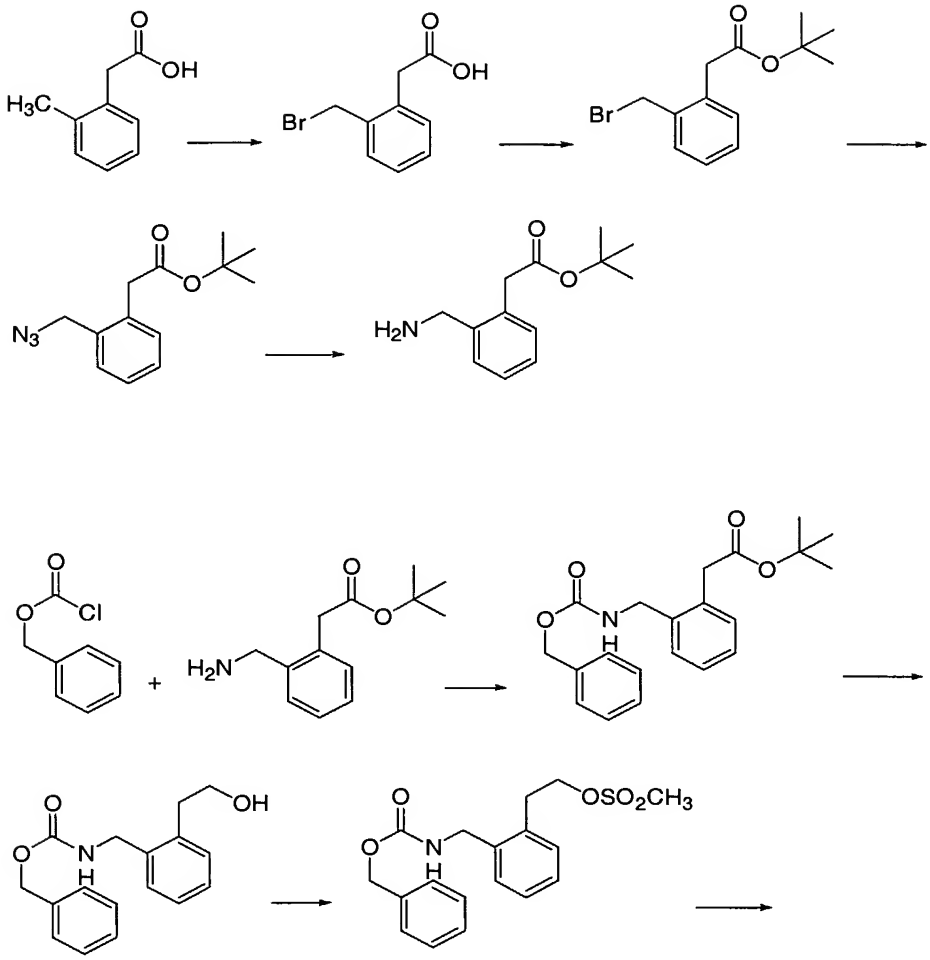
The compounds can also be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather than intermittent throughout the dosage regime.

The thrombin inhibitors are typically administered as active ingredients in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixers, syrups and the like, and consistent with convention pharmaceutical practices.

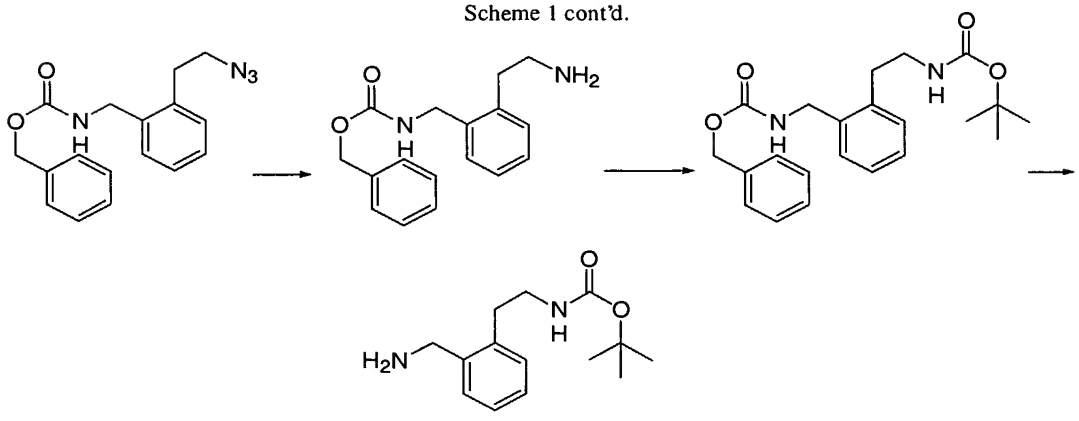
For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, distintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn-sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch methyl cellulose, agar, bentonite, xanthan gum and the like.

Unless otherwise stated, all NMR determinations were made using 400 MHz field strength.

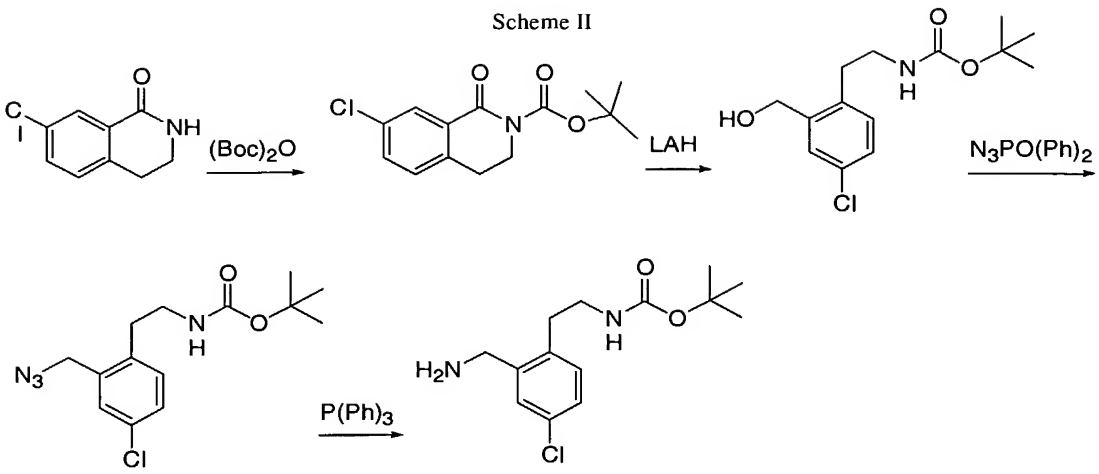
Scheme I



Scheme 1 cont'd.



Scheme II



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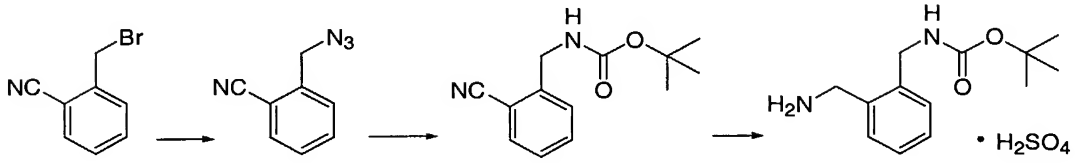
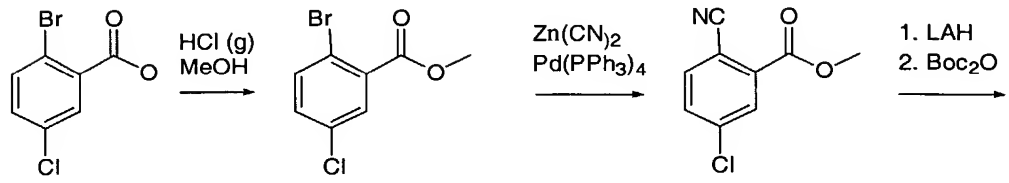
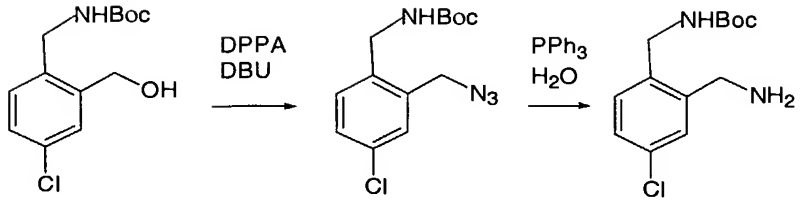
Scheme III



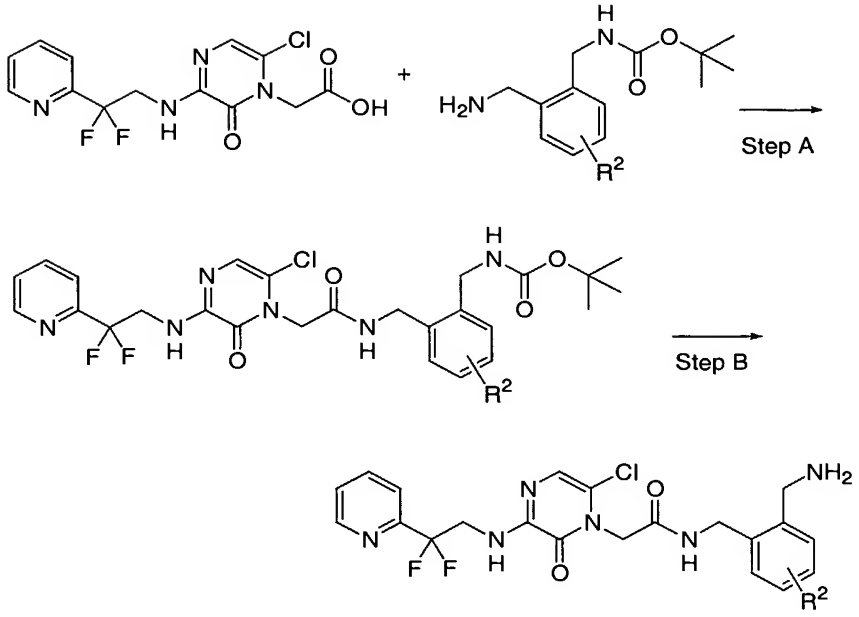
Scheme IV



Scheme IV cont'd



Scheme V



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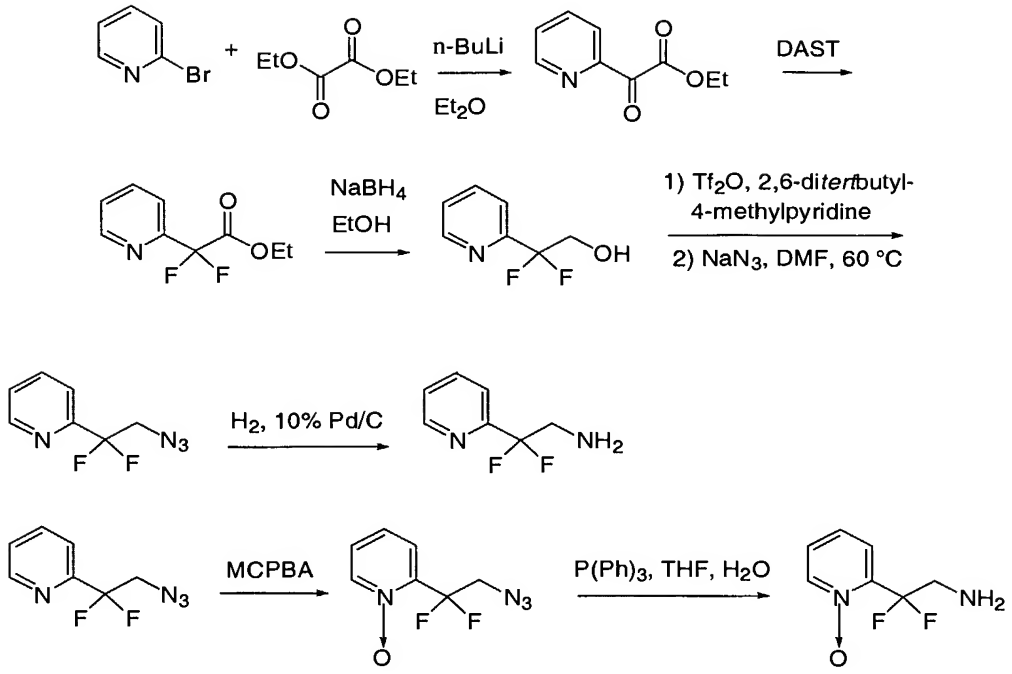
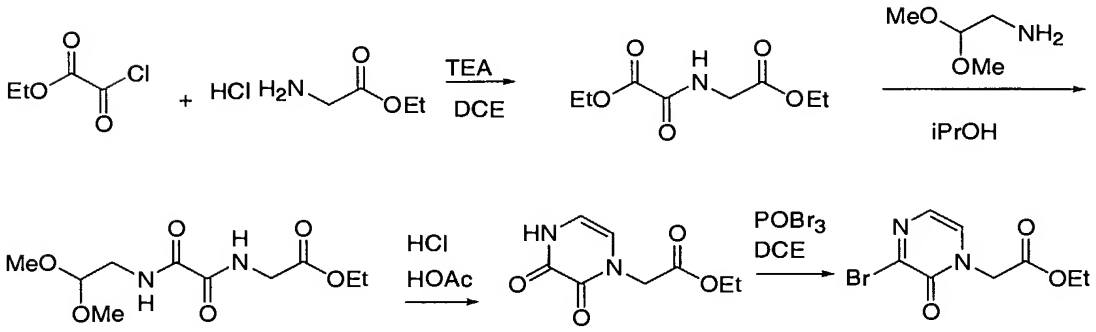
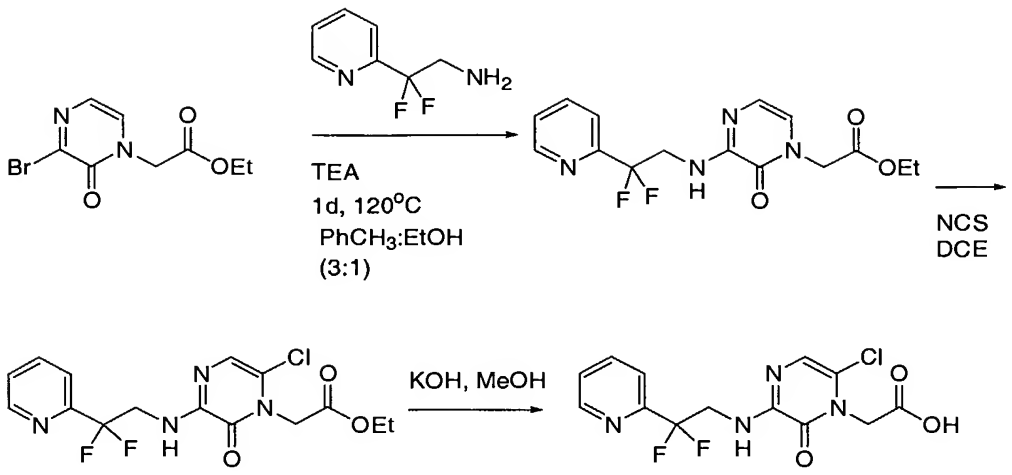
Scheme VI



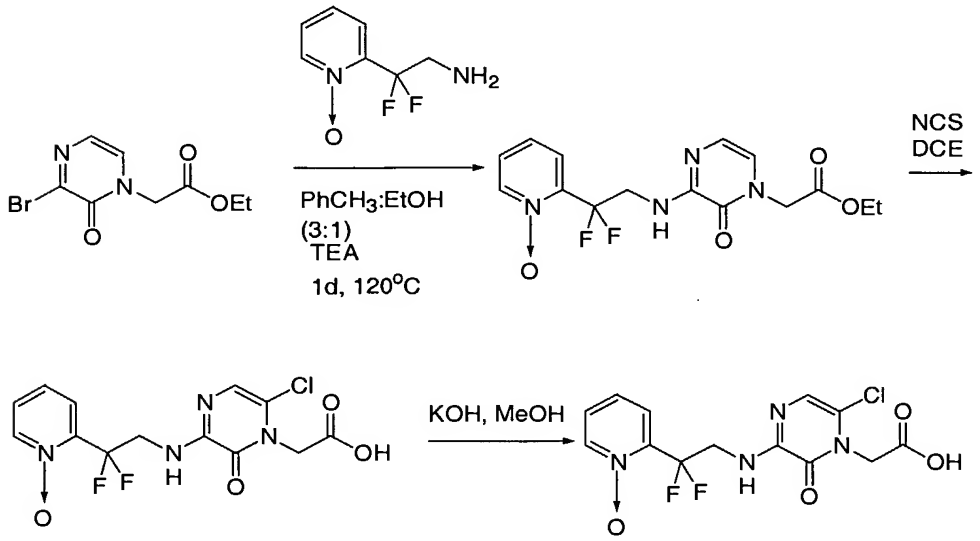
Scheme VII



Scheme VIII



Scheme IX



EXAMPLE 1

tert-Butyl 2-[2-(aminomethyl)phenyl]ethylcarbamate

Step A. 2-Bromomethylphenylacetic acid:

To a solution of 97.0 g (0.646 mol) *o*-tolylacetic acid in 1.75 L carbon tetrachloride was added 115.0 g (0.646 mol) *N*-bromosuccinimide and 3.4 g (0.021 mol) 2,2'-azobisisobutyronitrile. The mixture was heated at reflux under a nitrogen atmosphere for 4 h. After the mixture was cooled to 0-5 °C for 30 min, the solids were removed by filtration and washed with a small portion of carbon tetrachloride. This solid was triturated with water (0.8 L), collected on a filter, and washed with 500

mL of water to give 2-bromomethylphenylacetic acid. The filtrate was concentrated to a volume of 150 ml, and the resulting slurry cooled to 0-5 °C for 30 min. A second batch of product was obtained.

STEP B. 2-Bromomethylphenylacetic acid t-butyl ester:

To a solution of 80 g (0.349 mol) 2-bromomethylphenylacetic acid in 700 mL 1,4-dioxane in a 2 L heavy-walled flask was added 84 mL (1.571 mol) concentrated sulfuric acid at ambient temperature. The reaction mixture was chilled to –15 °C, and 580 mL isobutylene was condensed directly into the reaction vessel. The sealed pressure flask was shaken mechanically at room temperature for 4 h (the pressure inside the flask rises to ca. 20 psi during this step). The mixture was carefully quenched by slowly pouring it into a 0-5°C stirred mixture of 1.2 L tert-butyl methyl ether and 336 g (4.0 mol) solid sodium bicarbonate before slow dilution with 1.2 L ice-water. The separated organic phase was washed with 0.8 L brine, dried with sodium sulfate, filtered concentrated in vacuo to give an oil, which was used without further purification.

STEP C. 2-Azidomethylphenylacetic acid *t*-butyl ester:

To a solution of crude 2-bromomethylphenylacetic acid *t*-butyl ester (0.349 mol) in 600 mL DMF was added 34.1 g (0.524 mol) sodium azide and the mixture stirred at 65 °C for 3 h. After cooling to ambient temperature, the mixture was diluted with 1.2 L ethyl acetate. The organic layer was washed with water (3 x 800 mL), dried over sodium sulfate, filtered and concentrated in vacuo to give the title compound as a yellow oil. This material was used without further purification.

STEP D. tert-Butyl [2-(aminomethyl)phenyl]acetate oxalate salt:

To a solution of 78.0 g (0.312 mol) 2-azidomethylphenylacetic acid t-butyl ester in 1.36 L THF was added 7.8 g (50% water wet) 5 % Pd on C and the mixture shaken mechanically in a 2 L heavy-walled flask under H_2 at 45 psi for 2 h. The catalyst was removed by filtration through a bed of celite, rinsing with 250 mL THF. To the filtrate was added a solution of 31.25 g (0.347 mol) oxalic acid in 500 mL methyl tert-butyl ether, and the resultant suspension stirred at room temperature for 30 min. The solid was collected on a filter and washed with 300 mL methyl tert-butyl ether (the filtration was very slow, requiring about 3 hours). Drying under reduced pressure at 60°C for 18 h gave tert-butyl [2-(aminomethyl)phenyl]acetate oxalate salt as a white powder. The product is unstable as the free base, and will cyclize to the amide over several hours at room temperature.

STEP E. tert-Butyl [2-({[(benzyloxy)carbonyl]amino}methyl)phenyl]acetate:

To a solution of 3.0g (9.64mmol) tert-butyl [2-(aminomethyl)phenyl] acetate in 100mL methylene chloride cooled to 0°C was added 0.825ml (5.78mmol) benzyl chloroformate followed by 1.47g (12.05mmol) of 4-dimethylaminopyridine and a second 0.825ml (5.78mmol) portion

of benzyl chloroformate. After 30 min the reaction was washed with 10% potassium hydrogen sulfate (aq) (2x30mL), water (1x30mL), and brine (1x30mL), dried over sodium sulfate, filtered and concentrated to dryness in vacuo. The resulting crude oil (3.69g) was flash chromatographed on silica gel (15% ethyl acetate in hexane) to give tert-butyl [2-({[(benzyloxy)carbonyl] amino}methyl)phenyl]acetate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-7.30 (m, 5H), 7.28-7.21 (m, 4H), 5.40 (br s, 1H), 5.12 (s, 2H), 4.40 (d, 2H, J = 5.5Hz), 3.61 (s, 2H), 1.41 (s, 9H); MS (Electrospray): M+Na = 378.1; TLC R_f = 0.30 (15% ethyl acetate in hexane).

STEP F. Benzyl 2-(2-hydroxyethyl)benzylcarbamate:

To a solution of 3.15g (8.86mmol) tert-butyl [2({[(benzyloxy)carbonyl] amino}methyl)phenyl]acetate in 32mL of THF cooled to 0°C and under a nitrogen atmosphere was added dropwise 6.65mL of a 2.0M lithium borohydride solution in THF over 30 min. After stirring overnight at room temperature, the reaction was cooled in an ice bath and treated with 10% potassium hydrogen sulfate (aq) portionwise until fizzing subsided and extracted with ethyl acetate (3x). The organic extracts were combined, washed with brine (1x), dried over sodium sulfate, filtered and concentrated to dryness in vacuo to give 2.58g of a crude light yellow oil. Flash chromatography on silica gel (linear gradient from 5 to 40% ethyl acetate in hexane) gave benzyl 2-(2-hydroxyethyl)benzyl carbamate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37-7.19 (m, 9H), 5.44 (br s, 1H), 5.12 (s, 2H), 4.42 (d, 2H, J = 5.2Hz), 3.90-3.82 (br t, 2H), 2.95-2.88 (br t, 2H, J = 5.2Hz); MS (Electrospray): M+Na = 308.1; TLC R_f = 0.28 (40% ethyl acetate in hexane).

STEP G. 2-[2-({[(Benzyloxy)carbonyl]amino}methyl)phenyl]ethyl methanesulfonate:

To a solution of 1.06g (3.72mmol) benzyl 2-(2-hydroxyethyl)benzyl carbamate in 10mL of methylene chloride cooled to 0°C was added 0.570mL (4.09mmol) of triethylamine followed by 0.316mL (4.09mmol) of methanesulfonyl chloride. After stirring overnight at room temperature, the reaction was flash chromatographed directly on silica gel (40% ethyl acetate in hexane) to give 2-[2-({[(benzyloxy)carbonyl]amino}methyl)phenyl]ethyl methanesulfonate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-7.21 (m, 9H), 5.21 (br s 1H), 5.13 (s, 2H), 4.45-4.38 (m, 4H), 3.12 (br t, 2H, J = 6.7Hz), 2.84 (s, 3H); MS (Electrospray): M+Na = 386.0; TLC R_f = 0.35 (40% ethyl acetate in hexane).

STEP H. Benzyl 2-(2-azidoethyl)benzylcarbamate:

To a solution of 1.15g (3.16mmol) of 2-[2-({[(benzyloxy)carbonyl] amino}methyl)phenyl]ethyl methanesulfonate in 8.0mL of DMF was added 0.411g (6.33mmol) of sodium azide. After stirring at room temperature overnight, an additional 0.205g (3.15mmol) of sodium azide was added and the reaction warmed to 40°C for 4 h. The reaction was cooled to room

temperature, treated with saturated sodium carbonate (aq) and extracted with ethyl acetate (3x). The organic extracts were combined, washed with water (1x) and brine (1x), dried over sodium sulfate and concentrated to dryness in vacuo to give 1.2g of a crude oil. Flash chromatography on silica gel (20% ethyl acetate in hexane) gave benzyl 2-(2-azidoethyl)benzylcarbamate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.39-7.20 (m, 9H), 5.14 (s, 2H), 5.05 (br s, 1H), 4.42 (d, 2H, J = 5.6Hz), 3.56-3.49 (t, 2H, J = 7.0Hz), 2.97-2.91 (t, 2H, J = 7.0Hz); MS (Electrospray): M+Na = 333.1; TLC R_f = 0.32 (20% ethyl acetate in hexane).

STEP I. Benzyl 2-(2-aminoethyl)benzylcarbamate:

To a solution of 904mg (2.91mmol) benzyl 2-(2-azidoethyl)benzyl carbamate in 40.0mL THF containing 4.0mL water was added 1.53g (5.28mmol) triphenylphosphine and the reaction stirred at room temperature overnight. The THF was removed in vacuo and the residual aqueous phase extracted with methylene chloride (3x). The organics were combined, washed with brine (1x), dried over sodium sulfate, filtered and concentrated to dryness in vacuo. Flash chromatography on silica gel (linear gradient from 160/10/1 to 114/10/1 of methylene chloride/methanol/concentrated ammonium hydroxide) gave benzyl 2-(2-aminoethyl)benzylcarbamate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-7.17 (m, 9H), 6.14 (br s, 1H), 5.12 (s, 2H), 4.41 (d, 2H, J = 4.5Hz), 3.01-3.95 (t, 2H, J = 6.7Hz), 2.82-2.75 (t, 2H, J = 6.7Hz); MS (Electrospray): M+H = 285.1; TLC R_f = 0.18 (160/10/1 of methylene chloride/ methanol/concentrated ammonium hydroxide).

STEP J. Benzyl 2-{2-[(tert-butoxycarbonyl)amino]ethyl}benzylcarbamate:

To a solution of 584mg (2.05mmol) benzyl 2-(2-aminoethyl)benzyl carbamate in 6.0mL of methylene chloride at 0°C was added a 3.0mL methylene chloride solution of 493mg (2.26mmol) di-tert-butyldicarbonate. The reaction was stirred 0.5 h at 0°C and then warmed to room temperature for 2 h. Flash chromatography of the reaction directly onto silica gel (6% diethylether in methylene chloride) gave benzyl 2-{2-[(tert-butoxycarbonyl)amino]ethyl}benzylcarbamate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-7.15 (m, 9H), 5.56 (br s, 1H), 5.13 (s, 2H), 4.67 (br s 1H), 4.41 (d, 2H, J = 5.8Hz), 3.34-3.27 (m, 2H), 2.88-2.80 (br t, 2H, J = 7.0Hz), 1.42 (s, 9H); MS (Electrospray): M+Na = 407.1; TLC R_f = 0.42 (5% diethyl ether in methylene chloride).

STEP K. tert-Butyl 2-[2-(aminomethyl)phenyl]ethylcarbamate:

To a solution of 750mg (1.95mmol) benzyl 2-{2-[(tert-butoxycarbonyl) amino]ethyl}benzylcarbamate in 6.0mL absolute ethanol was added 150mg of 10% palladium on carbon catalyst. A balloon of hydrogen was bubbled into the stirring suspension at room temperature over 2 h. The reaction was filtered through celite and the filter pad washed with fresh absolute ethanol (2x). The filtrate was concentrated to dryness in vacuo to give tert-butyl 2-[2-(aminomethyl)phenyl]

ethylcarbamate as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31-7.19 (m, 4H), 5.68 (br s, 1H), 3.90 (s, 2H), 3.41-3.34 (br m, 2H), 2.90-2.83 (br t, 2H, J = 6.8Hz), 1.41 (s, 9H); MS (Electrospray): M+NH = 251.1; TLC R_f = 0.24 (160/10/1 of methylene chloride/methanol/concentrated ammonium hydroxide).

EXAMPLE 2

tert-Butyl 2-[2-(aminomethyl)-4-chlorophenyl]ethylcarbamate

Step A: 2-(tert-butoxycarbonyl)-7-chloro-3,4-dihydro-2H-isoquinolin-1-one

Into a stirred solution of 7-chloro-3,4-dihydroisoquinolin-1-one (11.6g, 12.6mMol) in 50mL of anhydrous N,N-dimethylformamide under inert atmosphere at ambient temperature was added diisopropylethylamine (17.0mL, 95.8mMol, 1.5eq), di-tert-butyl dicarbonate (15.33g, 70.26mMol, 1.1eq), and a catalytic amount of 4-(dimethylamino)pyridine. This was stirred at ambient temperature for 2 hours, concentrated in vacuo, then partitioned between methylene chloride and water. The organics were dried(Na_2SO_4) and concentrated in vacuo. The residue was purified by flash silica gel chromatography using methylene chloride as an eluent. Desired fractions were concentrated in vacuo to afford 2-(tert-butoxycarbonyl)-7-chloro-3,4-dihydro-2H-isoquinolin-1-one (2) as a clear colorless oil (hplc rt=3.55 min, method A; mass spec m/z=282.1).

Step B: 2-(tert-butoxycarbonylaminoethyl)-5-chlorobenzyl alcohol

Into a stirred solution of 2-(tert-butoxycarbonyl)-7-chloro-3,4-dihydro-2H-isoquinolin-1-one (18.4g, 64.38mMol) in 50mL of anhydrous tetrahydrofuran under inert atmosphere at 0°C was added 2.0M LiBH4 in tetrahydrofuran (64.38 mL, 128.76mMol, 2eq). This was stirred at 0°C for 1.5 hours, quenched with saturated ammonium chloride solution, then partitioned between ethyl acetate and water. The organics were dried(Na_2SO_4) and concentrated in vacuo. This afforded 2-(tert-butoxycarbonylaminoethyl)-5-chlorobenzyl alcohol as a clear colorless oil (hplc rt=3.21 min, method A; mass spec m/z=286.2).

STEP C. tert-Butyl 2-[2-azidomethyl)-4-chlorophenyl]ethylcarbamate:

To a solution of 649mg (2.27mmol) tert-butyl 2-[4-chloro-2-(hydroxymethyl)phenyl]ethylcarbamate in 5.0mL THF at 0°C was added 0.674mL (3.13mmol) of diphenylphosphoryl azide (DPPA) and 0.468mL (3.13mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and the reaction stirred at 0°C for 10 min, then at room temperature. After 3 h the reaction was treated with saturated sodium carbonate (aq) and extracted with ethyl acetate (3x). The organic extracts were combined, washed with brine (1x), dried over sodium sulfate, filtered and concentrated to dryness in vacuo to give 963mg of a crude oil. Flash chromatography on silica gel (15% ethyl acetate in hexane) gave tert-butyl 2-[2-azidomethyl)-4-chlorophenyl] ethylcarbamate as a colorless oil: ^{1}H NMR

(DMSO-d_6, 400 MHz): δ 7.44 (d, 1H, J = 1.8Hz), 7.36 (dd, 1H, J = 2.0 and 8.2Hz), 7.26 (d, 1H, J = 8.1Hz), 6.92 (br t, 1H, J = 5.5Hz), 4.53 (s, 2H), 3.10 (dt, 2H, J = 6.3 and 7.6Hz), 2.73 (t, 2H, J = 7.3Hz), 1.36 (s, 9H); MS (Electrospray): M+Na = 333.0; TLC R_f = 0.32 (15% ethyl acetate in hexane).

STEP D. tert-Butyl 2-[2-(aminomethyl)-4-chlorophenyl]ethylcarbamate:

To a solution of 629mg (2.02mmol) tert-butyl 2-[2-azidomethyl)-4-chlorophenyl]ethylcarbamate in 30.0mL THF containing 3.1mL water was added 1.06g (4.05mmol) triphenylphosphine and the reaction stirred at room temperature overnight. The THF was removed in vacuo and the residual aqueous phase extracted with methylene chloride (3x). The organics were combined, washed with brine (1x), dried over sodium sulfate, filtered and concentrated to dryness in vacuo. Flash chromatography on silica gel (linear gradient from 266/10/1 to 200/10/1 of methylene chloride/methanol/concentrated ammonium hydroxide) gave tert-butyl 2-[2-(aminomethyl)-4-chlorophenyl]ethylcarbamate as a colorless oil: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.45 (d, 1H, J = 1.7Hz), 7.18 (dd, 1H, J = 2.1 and 8.2Hz), 7.12 (d, 1H, J = 8.2Hz), 7.01 (br t, 1H, J = 5.2Hz), 3.73 (s, 2H), 3.07 (dt, 2H, J = 6.5 and 7.3Hz), 2.68 (t, 2H, J = 7.4Hz), 1.36 (s, 9H); MS (Electrospray): M+H = 285.1; TLC R_f = 0.33 (160/10/1 of methylene chloride/methanol/concentrated ammonium hydroxide).

EXAMPLE 3

tert-Butyl 2-(aminomethyl)benzylcarbamate

STEP A. 2-(Azidomethyl)benzonitrile:

To a solution of 90 g (459 mmol) 2-cyanobenzylbromide in 600 mL THF was added in one portion a solution of 36 g (553 mmol) sodium azide in 100 mL water. The two phase mixture was stirred at 23°C for 18 hr. The THF layer was separated from the lower water layer and used in the next step without further purification.

STEP B. tert-Butyl 2-cyanobenzylcarbamate:

To the THF layer from the previous step was diluted to a volume of 1.6 L, divided into two equal portions and each hydrogenated at 45psi in a Parr pressure bottle containing 6 g of 5% palladium on carbon (50% water by weight). A 5-10°C exotherm was observed within 30 min and shaking continued a total of 1.5 hr. The individual batches were filtered through celite, washed 2x with 100 mL fresh THF and the filtrates combined into a single portion. To the amine mixture without concentration (Caution: attempts to concentrate the solution resulted in a large exotherm and the batch turned black) was added 87.5 mL (381 mmol) of di-tert-butyl dicarbonate neat. After 2 hr the THF was

removed in vacuo and flushed with 250 mL of 15% ethyl acetate in hexane. The semi-solid was slurried in 250 mL of 15% ethyl acetate and filtered. The filtrate was concentrated in vacuo, diluted with 10% ethyl acetate in hexane (175 mL), cooled to 0°C and filtered to give tert-butyl 2-cyanobenzylcarbamate as a gray solid.

STEP C. tert-Butyl 2-(aminomethyl)benzylcarbamate hemisulfate:

To a 3 L, 3 neck flask fitted with a thermocouple, a condenser and nitrogen inlet was added 3 g (23 mmol) of cobaltous chloride, then 1200 mL of THF followed by 59 g (254 mmol) of tert-butyl 2-cyanobenzylcarbamate and 600 mL of ice-water. To the light pink solution at 15°C was added 26 g (684 mmol) of sodium borohydride in portions as follows. The initial 3 g of sodium borohydride resulted in a vigorous hydrogen gas evolution and formation of a black suspension. The batch temperature reached 35°C within 2 hr, and was maintained at this temperature with a heating mantle. Additional sodium borohydride and cobaltous chloride were added as needed to drive the reaction to completion. Typically, 2 x 7.5 g of additional sodium borohydride and 2 x 1 g portions of cobaltous chloride were added at 12 hour intervals. Once complete, the layers were allowed to settle and the clear upper THF layer was decanted from the black aqueous layer. The aqueous layer was washed with 750 mL fresh THF, the two THF layers combined and filtered through a pad of celite. The orange-yellow filtrate was concentrated to about 300 mL in vacuo, resulting in water layer with the product as an oily lower layer. The mixture was extracted with 2 x 250 mL ethyl acetate and the combined extracts reacted with 24 g (200 mmol) of solid sodium hydrogensulfate. A solid formed immediately, and the slurry was stirred for 30 min, filtered and washed with 2 x 100 mL ethyl acetate to give 62 g of a white powder. The powder was slurried in 175 mL water, cooled to 0°C, filtered, washed with 2 x 40 mL cold water and the solid dried in a vacuum oven at 55°C for 24 hr to give tert-butyl 2-(aminomethyl)benzylcarbamate hemisulfate salt as a white powder.

EXAMPLE 4

tert-Butyl 2-(aminomethyl)-4-chlorobenzylcarbamate

Step A. 2-bromo-5-chlorobenzoate

Through a solution of 2-bromo-chlorobenzoic acid (11g, 46.7 mmol) in methanol (250ml) was bubbled HCl gas. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture is concentrated in vacuo to give an orange oil, which is purified by flash chromatography (silica gel, hexane) to give the title compound as a colorless oil.

1H NMR ($CDCl_3$, 400MHz): δ 7.78 (d, 1 H, J= 2.6 Hz); 7.59 (d, 1H, J= 12.81 Hz);7.30 (dd, 1 H, J= 8.6, 2.5 Hz); 3.94 (s, 3H)

Step B. Methyl 5-chloro-2-cyanobenzoate

To a solution of methyl 2-bromo-5-chlorobenzoate (1.15g, 4.6 mmol) in degassed DMF was added zinc cyanide (282 mg, 2.40 mmol) and palladium tetrakis triphenylphosphine (100mg, 0.086 mmol) and the reaction is stirred at 90C over night. The reaction was partitioned between ethyl acetate and water. The organic was concentrated in vacuo and purified by flash chromatography eluting a gradient to 10 to 25% ethyl acetate in hexane yielding a white solid (methyl 5-chloro-2-cyanobenzoate).

H NMR ($CDCl_3$, 400 MHz): δ 8.13 (d, 1 H, J= 1.83 Hz); 3.09 (d, 1 H, J= 8.24 Hz); 7.29 (dd, 1 H, J= 8.34, 2.10 Hz); 4.02 (s, 3 H)

Step C. [2-(aminomethyl)-5-chlorophenyl]methanol

To LAH (1 M/Et_2O, 104.4 ml, 104.4 mmol) in anhydrous THF (300 ml) at 0C was added methyl 5-chloro-2-cyanobenzoate (9.28g, 0.512 mmol) maintaining the temperature below 20 C.. After one half hour, quenched at OC with water (3.97 ml), NaOH (1N, 11.9 ml, 11.9 mmol) and water (3.97 ml). A precipitate was filtered out and washed with THF. The filtrate was concentrated in vacuo and was used immediately in the next step.

H NMR ($CDCl_3$, 400 MHz): δ 7.17-7.36 (m, 3 H); 4.60 (s, 2 H); 3.98 (s, 2 H);

Step D. *tert*-butyl 4-chloro-2-(hydroxymethyl)benzylcarbamate

To a solution of [2-(aminomethyl)-5-chlorophenyl]methanol in dichloromethane (200ml), was added di-tert-butyl-dicarbonate (11.38 g, 52.18 mmol) at room temperature. After one hour, the reaction was partitioned. The organic layer was concentrated in vacuo and purified by flash chromatography eluting a gradient of ethyl acetate/hexane which gave a brown oil, which was taken up in dichloromethane (500 ml) and treated with activated charcoal yielding a pink solid.

H NMR ($CDCl_3$, 400 MHz): 7.36 (s, 1 H); 7.2-7.5 (m, 2 H); 4.69 (b s, 2 H); 4.32 (d, 2 H, J= 6.04 Hz); 1.43 (s, 9 H).

Step E. *tert*-Butyl 2-(azidomethyl)-4-chlorobenzylcarbamate

To a solution of *tert*-butyl 4-chloro-2-(hydroxymethyl)benzylcarbamate (10 g, 36.8 mmol) in anhydrous THF (100 ml) was added DPPA (8.3 ml, 38.6 mmol) and DBU (5.79 ml, 38.6 mmol). The mixture was stirred overnight and then was partitioned between ethyl acetate and water. The organic layer was washed with brine, and was concentrated in vacuo to a crude oil (14.6 g). Purification was accomplished by silica gel chromatography, eluting a gradient of ethyl acetate-hexane (10, 15, 20, 25, 50%) to give *tert*-butyl 2-(aminomethyl)-4-chlorobenzylcarbamate.

H NMR ($CDCl_3$, 400 MHz): δ 7.25-7.39 (m, 3 H); 4.41 (s, 2 H), 4.32 (d, 2 H, J= 5.86 Hz); 1.45 (s, 9 H).

Step F. *tert*-Butyl 2-(aminomethyl)-4-chlorobenzylcarbamate

To a solution of *tert*-butyl 2-(azidomethyl)-4-chlorobenzylcarbamate (10.9 g, 36.73 mmol) in THF (60 ml) and water (6 ml) was added triphenylphospine (10.59 g, 40.40 mmol). The reaction was heated to 65 C and stirred overnight at room temperature. The reaction was concentrated in vacuo and flashed with 4% (10%NH4OH/MeOH)/ dichlor-omethane. A second purification using silica gel column chromatography with a careful gradient of 3 to 5% (10%NH4OH /MeOH)/ dichloro methane gave the title compound.

H NMR ($CDCl_3$, 400 MHz) δ 7.21-7.52 (m, 3 H); 4.32 (b d, 2 H); 3.90 (s, 2 H); 1.44 (s, 9 H).

EXAMPLE 5

3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-chloropyrazin(1H)-2-one-1-acetic acid

Step A. Ethyl 2-pyridinoylformate

To a stirred solution of 20 mL (210 mmol) of 2-bromopyridine in 500 mL of dry ether at -78°C under Ar was added 85 mL of a 2.5 M solution of n-butyllithium in hexane in a slow stream. After stirring in the cold for 30 min, the solution was transferred over a 5 min period via two cannula into a 0°C stirred solution of 100 mL (736 mmol) of diethyl oxalate in 1.0 L of dry ether under Ar. After stirring for 2h in the cold, the reaction mixture was washed with 600 mL of sat. $NaHCO_3$, water, and brine. The solution was dried over $MgSO_4$ and the solvents concentrated at reduced pressure to give a red oil that was purified by SiO_2 chromatography (10 x 15 cm) using 1:4 to 35:65 EtOAc-hexanes. The product-containing fractions were concentrated at reduced pressure to afford the product as a reddish oil: 1H NMR (CDCl3) δ 1.42 (t, 3H), 4.45-4.55 (m, 2H), 7.55-7.6 (m, 1H), 7.9-7.95 (m, 1H), 8.11 (d, 1H), 8.78 (d, 1H).

Step B. Ethyl difluoro-2-pyridylacetate

A stirred solution of 22 g (123 mmol) of ethyl 2-pyridinoylformate and 75 g (465 mmol) of diethylaminosulfurtrifluoride (DAST) were heated to 55°C under Ar overnight. Because the reaction was not complete, 5 g additional DAST was added, and the reaction heated for an additional 24 h. The reaction mixture was cooled to rt, and poured very slowly into a stirred mixture of 1 kg of ice, 400 mL of ethyl acetate and 500 mL of sat. $NaHCO_3$. After the addition, the mixture was basified by the addition of solid $NaHCO_3$. The aqueous layer was extracted with EtOAc, and the combined organic layers washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4 and the solvents concentrated at reduced pressure to give the product as a brown oil: ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H), 4.35-4.4 (m, 2H), 7.4-7.45 (m, 1H), 7.75 (d, 1H), 7.95 (d, 1H), 8.45 (d, 1H).

Step C. 2,2-Difluoro-2-(2-pyridyl)ethanol

To a stirred solution of 19.5 g (97 mmol) of ethyl difluoro-2-pyridylacetate in 200 mL of absolute ethanol at 0°C was added 4.42 g (116 mmol) of sodium borohydride in small portions. After 30 min, the reaction was quenched by the addition of 50 mL of sat. NH_4Cl. The reaction mixture was concentrated at reduced pressure and the residue partitioned between 500 mL of ethyl acetate and sat. $NaHCO_3$. The organic layer was washed with water, brine, and dried over Na_2SO_4 and concentrated at reduced pressure to give a brown oil that was purified on SiO_2 (10 x 17 cm) using 1:1 EtOAc-hexane. After re-chromatographing the mixed fractions, all clean fractions were combined and concentrated at reduced pressure, giving the product as a beige crystalline solid: ^1H NMR ($CDCl_3$) δ 3.6 (t, 1H), 4.17-4.3 (m, 2H), 7.4-7.45 (m, 1H), 7.73 (d, 1H), 7.84-7.91 (m, 1H), 8.61 (d, 1H).

Step D. 2,2-Difluoro-2-(2-pyridyl)ethyl trifluoromethanesulfonate

To a stirred solution of 5 g (31.4 mmol) of 2,2-difluoro-2-(2-pyridyl)ethanol and 9.69 g (47.2 mmol) of 2,6-di-t-butyl-4-methylpyridine in 110 mL of methylene chloride at -78°C under Ar was added 7.93 mL (47.2 mmol) of triflic anhydride dropwise. After 1h, the reaction was diluted with 100 mL of pentane and filtered. The filtrate was concentrated and treated again with pentane and filtered. Concentration of the filtrate gave the product as a brown oil, contaminated with 2,6-di-t-butyl-4-methylpyridine: ^1H NMR ($CDCl_3$) δ 5.12 (t, 2H), 7.45-7.5 (m, 1H), 7.75 (d, 1H), 7.86-7.94 (m, 1H), 8.65 (d, 1H).

Step E. 2,2-Difluoro-2-(2-pyridyl)ethylazide

To a stirred solution of 5.5 g of 2,2-difluoro-2-(2-pyridyl)ethyl trifluoromethanesulfonate 1-4 in 70 mL of DMF was added 6.74 g (104 mmol) of sodium azide under Ar. The mixture was heated to 60°C overnight. A second batch was run in the same manner, and after cooling to rt, both reactions were poured into 600 mL of water, and extracted with 3 x 500 mL of ether. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated at reduced pressure to give an oil that was purified by SiO_2 (10 x 6 cm) using hexane 1:3 EtOAc-hexane and 1:1 EtOAc-hexane. The product-containing fractions were concentrated at reduced pressure to give the product as a yellow oil: ^1H NMR ($CDCl_3$) δ 4.05 (t, 2H), 7.4-7.45 (m, 1H), 7.73 (d, 1H), 7.83-7.89 (m, 1H), 8.67 (d, 1H).

Step F. 2,2-Difluoro-2-(2-pyridyl)ethylamine

A stirred solution of 100 mg of 2,2-difluoro-2-(2-pyridyl)ethylazide was hydrogenated in 10 mL of ethyl acetate over 100 mg of 10% palladium on carbon using a balloon for 1 h. The catalyst was removed by filtration and the solvents removed at reduced pressure. A total of 1.8 g (9.7 mmol) of the azide was reduced using this procedure to give 2,2-difluoro-2-(2-

pyridyl)ethylamine as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.66 (d, 1H, 4.2 Hz), 7.82 (td,1H, 7.7, 1.7 Hz), 7.68 (d, 1H, 8.1 Hz), 7.37-7.40 (m, 1H), 3.44 (t, 2 H, 14.3 Hz), 1.41 (br s, 2H).

Step G. Ethyl N-(ethyl carboxymethyl)oxamate

To a suspension of ethyl glycine•HCl (38.4 g, 275 mmol) in 1,2-dichloroethane (360 mL) was added triethylamine (77.0 mL, 550 mmol) at room temperature. After stirring for 30 minutes the heterogenous mixture was cooled to 0 °C and ethyl oxalyl chloride (30.3 mL, 275 mol) was added dropwise over the course of 1 h. Upon completion of the addition, the cooling bath was removed and the reaction was stirred at room temperature overnight. The reaction was diluted with water (250 mL) and the layers separated. The aqueous layer was backwashed with 2 portions of dichloromethane (250 mL). The combined organic layers were washed with water (250 mL), followed by brine (250 mL), dried over $MgSO_4$ and concentrated to give ethyl n-(ethyl carboxymethyl)oxamate as an oil that was taken directly onto the next step.

Step H. N-(Ethyl carboxymethyl)-N'-(2,2-dimethoxyethyl)oxamide

To a solution of the oxamate (84.0 g, 414 mmol) 2-1 in 2-propanol (500 mL) was added aminoacetaldehyde dimethyl acetal (45.7 g, 435 mmol) in one portion. After stirring overnight at room temperature, the reaction mixture was concentrated to a thick orange oil. This thick slurry was diluted with 2-propanol (300 mL) and the solid was broken up with a spatula. Filtration afforded a solid which was further rinsed with an additional portion of 2-propanol. Removal of residual 2-propanol was accomplished via high vacuum to afford a light orange solid: ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1H), 7.50 (br s, 1H), 4.41 (t, 1H, 5.3 Hz), 4.24 (q, 2H, 7.1 Hz), 4.09 (d, 2H, 5.9 Hz), 3.47 (dd, 2H, 5.3, 6.2 Hz), 3.40 (s, 6H), 1.30 (t, 3 H, 7.1 Hz).

Step I. Ethyl 3-hydroxypyrazin(1H)-2-one-1-acetate

A solution of the oxamide (89.8 g, 343 mmol), acetic acid (400 mL), and conc. HCl (2 mL) was heated to reflux. After 1 h the black reaction was concentrated to a thick oil (high vacuum employed to ensure complete removal of AcOH) which was diluted with EtOH (150 mL) and MeOH (150 mL). Scraping the thick black oil with a spatula induced precipitation of the product. The MeOH was removed via rotary evaporation and the remaining slurry was filtered and rinsed with EtOH (200 mL) to deliver a tan solid. Recrystallization from refluxing EtOH (300 mL) afforded the product as an off-white powder: ^{1}H NMR (CD_3OD) δ 6.50 (d, 1H, 5.9 Hz), 6.36 (d, 1H, 5.9 Hz), 4.58 (s, 2H), 4.23 (q, 2H, 7.1 Hz), 1.28 (t, 3 H, 7.1 Hz). Further crude dione could be obtained upon concentration of the mother liquor.

Step J. Ethyl 3-bromopyrazin(1H)-2-one-1-acetate

A solution of the hydroxypyrazinone (25.0 g, 126 mmol) and phosphorous oxybromide (37.9 g, 132 mmol) in 1,2-dichloroethane (250 mL) was heated to reflux. After 8 h the reaction mixture was treated with sat. aq. Na_2CO_3 (250 mL) and stirred for 1h. The mixture was diluted with water (100 mL) and dichloromethane (100 mL), the layers were separated and the aqueous layer was backwashed with EtOAc (3 x 200mL). The combined organics were dried ($MgSO_4$), and concentrated to give an oil which was stored on a high vacuum line overnite to afford brown solid. ^1H NMR ($CDCl_3$) δ 7.17 (d, 1H, 4.2 Hz), 7.07 (d, 1H, 4.2 Hz), 4.65 (s, 2H), 4.27 (q, 2H, 7.2 Hz), 1.31 (t, 3 H, 7.2 Hz).

Step K. Ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)pyrazin(1H)-2-one-1-acetate

A solution of 4.80 g (30.4 mmol) of 2,2-difluoro-2-(2-pyridyl)ethylamine, 4.24 mL (30.4 mmol) of triethylamine and 7.93 g (30.4 mmol) of ethyl 3-bromopyrazin(1H)-2-one-1-acetate was heated to 120 °C in a sealed tube overnight in 12 mL of toluene and 4 mL of ethanol. The reaction was concentrated and the residue was partitioned between dichloromethane and sat. aq. $NaHCO_3$. The aqueous layer was backwashed with 4 portions of dichloromethane. The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure to give an oil that was chromatographed on SiO_2 using 60:40 to 40:60 hexane-EtOAc to give Ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)pyrazin(1H)-2-one-1-acetate as a yellow solid: ^1H NMR ($CDCl_3$) δ 8.67 (dd, 1H, 4.8, 0.7 Hz), 7.81 (ddd,1H, 7.8, 7.8, 1.7 Hz), 7.69 (dd, 1H, 7.8, 1 Hz), 7.38 (dd, 1H, 5.1, 7.0 Hz), 6.86 (d, 1H, 4.8 Hz), 6.54 (br t, 1H, 5.9 Hz), 6.40 (d, 1H, 4.6 Hz), 4.54 (s, 2H), 4.38 (td, 2H, 14.0, 6.4 Hz), 4.24 (q, 2H, 7.1 Hz), 1.29 (t, 3 H, 7.1 Hz).

Step L. Ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)-6-chloropyrazin(1H)-2-one-1-acetate

A stirred solution of 6.81 g (20.1 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)pyrazin(1H)-2-one-1-acetate and 2.42 g (18.1 mmol) of N-chlorosuccinimide in 100 mL of 1,2-dichloroethane was heated to reflux. An additional 242 mg (1.81 mmol) and 75 mg (0.56 mmol) of NCS were added to the reaction mixture after 1 h and 1.5 h, respectively. After 2.5 h total, the solution was cooled to room temperature and partitioned between dichloromethane (150 mL) and sat. aq. $NaHCO_3$ (200 mL). The layers were separated and the aqueous phase was backwashed with dichloromethane (2 x 200 mL). The combined organic layers were dried over $MgSO_4$ and the solution concentrated to a volume of 10 mL. This liquid was directly loaded onto a SiO_2 column and eluted with 65:35 to 55:45 hexane-EtOAc to give the title compound as a yellow solid: ^1H NMR ($CDCl_3$) δ 8.68 (d, 1H, 4.8, Hz), 7.83 (ddd,1H, 7.7, 7.7, 1.6 Hz), 7.9 (dd, 1H, 7.9 Hz), 7.40 (dd, 1H, 4.9, 7.3 Hz), 6.96 (s, 1H), 6.49 (br t, 1H, 5.9 Hz), 4.89 (s, 2H), 4.38 (td, 2H, 13.9, 6.5 Hz), 4.26 (q, 2H, 7.1 Hz), 1.30 (t, 3 H, 7.1 Hz).

Step M. 3-(2,2-Difluoro-2-(2-pyridylethylamino)-6-chloropyrazin(1H)-2-one-1-acetic acid

To a stirred solution of 7.27 g (19.5 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridylethylamino)-6-chloropyrazin(1H)-2-one-1-acetate in 200 mL of methanol was added 39 mL (39.0 mmol) of 1M aq. potassium hydroxide. After 3 h the solution was acidified to pH = 7 using conc. HCl, and concentrated at reduced pressure (azeotrope with $PhCH_3$) to give a white solid containing potassium chloride and the product. ^1H NMR (CD_3OD) δ 8.64 (d, 1H, 4.8 Hz), 7.93 (ddd,1H, 7.7, 7.7, 1.5 Hz), 7.70 (d, 1H, 8.0 Hz), 7.49 (dd, 1H, 5.2, 7.4 Hz), 6.80 (s, 1H), 4.67 (s, 2H), 4.27 (t, 2H, 13.9 Hz).

EXAMPLE 6

3-(2,2-Difluoro-2-(2-pyridyl-N-oxide-ethylamino)-6-chloropyrazin(1H)-2-one-1-acetic acid

Step A 2,2-Difluoro-2-(2-pyridyl-N-oxide)ethylazide

To a stirred solution of 2,2-difluoro-2-(2-pyridyl-N-oxide)ethylazide (5.75 g, 31.3 mmol) in 1,2-dichloroethane (100 mL) was added 3-chloroperoxybenzoic acid (10.26 g, 41.6 mmol) and 3-tert-butyl-4-hydroxy-5-methylphenyl sulfide (1.12 g, 3.13 mmol) under Ar. The mixture was heated at 55 °C overnight. In the morning, the solution was poured into a sat. aq. $NaHCO_3/Na_2S_2O_3$ solution (200 mL). The layers were separated and the aqueous phase was backwashed with dichloromethane (3 x 150 mL). The combined organic layers were dried over $MgSO_4$, concentrated and chromatographed on a short SiO_2 column using 100% EtOAc to give the title compound as a white solid: ^1H NMR ($CDCl_3$) δ 4.38 (t, 2H, 13.5 Hz), 7.36-7.44 (m, 2H), 7.72 (dd, 1H, 2.3 Hz, 7.6 Hz), 8.26 (d, 1H, 6.1 Hz).

Step B 2,2-Difluoro-2-(2-pyridyl-N-oxide)ethylamine

Triphenylphosphine (7.72 g, 29.5 mmol) was added to a water bath cooled solution of 2,2-difluoro-2-(2-pyridyl-N-oxide)ethylazide (5.61 g, 28.1 mmol) in THF (90 mL). After 1 h water (10 mL) was added and the mixture was heated to 55 °C. Two hours after the addition of water, the heating bath was removed and the solution was allowed to stir overnight. The reaction was subsequently concentrated, diluted with EtOAc (250 mL), and HCl (25 mL, 2.6M in EtOAc) was added dropwise. Stirring was continued for 20 min, after which time the mixture was filtered and rinsed with EtOAc (150 mL). To a stirred suspension of this solid in dichloromethane (300 mL) was added NaOH (3.33 g in 15 mL H_2O) dropwise. After 15 min the mixture was poured into a separatory funnel and the organic phase was separated. The aqueous phase was saturated with solid NaCl and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to an oil which solidified upon storage in the: ^1H NMR ($CDCl_3$) δ 8.25 (br d, 1H, 6.2 Hz), 7.69 (dd, 1H, 2.8, 7.3 Hz), 7.32-7.39 (m, 2H), 3.76 (t, 2 H, 15.2 Hz), 1.29 (br s, 2H).

Step C Ethyl 3-(2,2-difluoro-2-(2-pyridyl-N-oxide-ethylamino)pyrazin(1H)-2-one-1-acetate

A mixture of 3.0 g (17.2 mmol) of 2,2-difluoro-2-(2-pyridyl-N-oxide)ethylamine, 2.72 mL (19.5 mmol) of triethylamine and 4.5 g (17.2 mmol) of ethyl 3-bromopyrazin(1H)-2-one-1-acetate in 9 mL of toluene and 3 mL of ethanol was heated to 120 °C in a sealed tube for 24 h. The reaction was concentrated and the residue was partitioned between EtOAc (200 mL) and sat. aq. $NaHCO_3$ (200 mL). The aqueous layer was backwashed with EtOAc (5 x 150 mL). The combined organic layers were dried over $MgSO_4$ and the solvents removed at reduced pressure to give a brown solid. This crude material was diluted with EtOAc (50 mL), filtered, and rinsed with EtOAc (2 x 50 mL) to afford the title compound as a tan powder: ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1H, 6.4 Hz), 7.61 (br d, 1H, 7.9 Hz), 7.34 (dd, 1H, 6.6, 6.6 Hz), 7.26 (dd, 1H), 6.78 (d, 1H, 4.6 Hz), 6.39 (br t, 1H, 6.6 Hz), 6.37 (d, 1H, 4.6 Hz), 4.66 (td, 2H, 13.8, 7.0 Hz), 4.52 (s, 2H), 4.23 (q, 2H, 7.1 Hz), 1.28 (t, 3 H, 7.1 Hz).

Step D Ethyl 3-(2,2-difluoro-2-(2-pyridyl-N-oxide-ethylamino)-6-chloropyrazin(1H)-2-one-1-acetate

A stirred solution of 4.96 g (14.0 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridyl-N-oxide-ethylamino)pyrazin(1H)-2-one-1-acetate and 1.86 g (14.0 mmol) of N-chlorosuccinimide in 200 mL of 1,2-dichloroethane was heated to 70 °C. After 3 h the solution was cooled to room temperature and partitioned between dichloromethane (150 mL) and sat. aq. $NaHCO_3$ (200 mL). The layers were separated and the aqueous phase was backwashed with dichloromethane (4 x 200 mL) and EtOAc (2 x 200 mL). The combined organic layers were dried over $NaSO_4$ and the solution concentrated. This crude solid was purified on a SiO_2 column with 100 % EtOAc to 10:90 MeOH:EtOAc to give the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1H, 6.4 Hz), 7.62 (dd, 1H, 2.2, 7.9 Hz), 7.35 (ddd, 1H, 2.1, 7.7, 7.7 Hz), 7.2 (dd, 1H, 7.7 Hz), 6.86 (s, 1H), 6.35 (br t, 1H, 6.7 Hz), 4.85 (s, 2H), 4.64 (td, 2H, 13.8, 6.9 Hz), 4.24 (q, 2H, 7.1 Hz), 1.29 (t, 3 H, 7.1 Hz).

Step E 3-(2,2-Difluoro-2-(2-pyridyl-N-oxide-ethylamino)-6-chloropyrazin(1H)-2-one-1-acetic acid

To a stirred solution of 4.88 g (12.6 mmol) of ethyl 3-(2,2-difluoro-2-(2-pyridyl-N-oxide-ethylamino)-6-chloropyrazin(1H)-2-one-1-acetate in methanol (100 mL) was added 5.0 g potassium hydroxide (89.1 mmol dissolved in 20 mL water). After 1 h the solution was concentrated, diluted with 50 mL of water and acidified to pH = 7 using conc. HCl. Concentration at reduced pressure (azeotrope with $PhCH_3$) afforded an off-white solid containing potassium chloride and the title compound: ^{1}H NMR (CD_3OD) δ 8.36 (d, 1H, 6.2 Hz), 7.69 (dd, 1H, 7.7, 2.2 Hz), 7.51- 7.59 (m, 2H), 6.67 (s, 1H), 4.62 (s, 2H), 4.55 (t, 2H, 13.1 Hz).

EXAMPLE 7

[3-[(2,2-Difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid:

To a solution of 2.84 g (8.41 mmol) ethyl 3-(2,2-difluoro-2-(2-pyridyl)ethylamino)-pyrazin(1H)-2-one-acetate in 10.0 mL methanol was added 8.83 mL (8.83 mmol) 1.0N lithium hydroxide (aq) and the light yellow solution stirred 1 h at room temperature. The reaction pH was adjusted to 7.0 with a dropwise addition of 1.0N HCl in ether to give a precipitate. The solid was removed by filtration, washed well with water and dried under high vacuum for 24 h to give [3-[(2,2-Difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.13 (br s, 1H), 8.70 (d, 1H, *J* = 4.7Hz), 7.98 (t, 1H, *J* = 7.8Hz), 7.70 (d, 1H, *J* = 7.9Hz), 7.60-7.55 (m, 1H), 7.16 (t, 1H, *J* = 6.7Hz), 6.84 (d, 1H, *J* = 4.7Hz), 6.73 (d, 1H, *J* = 4.6Hz), 4.57 (s, 2H), 4.24 (dt, 2H, *J* = 6.6 and 15.2Hz); MS (Electrospray): M+H = 311.0.

EXAMPLE 8

3-(2,2-Difluoro-2-(2-pyridyl-N-oxide-ethylamino)- pyrazin(1H)-2-one-1-acetic acid

Prepared from Ethyl 3-(2,2-difluoro-2-(2-pyridyl-N-oxide-ethylamino)pyrazin(1H)-2-one-1-acetate by a procedure similar to that described above for Example 7:
MS (Electrospray): M+H = 327.0.

EXAMPLE 9

Step A: tert-Butyl 2-[({[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetyl}amino)methyl]benzylcarbamate:

To a solution of 500 mg (1.16 mmol) [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and 465 mg (1.39 mol) tert-butyl 2-(aminomethyl)benzylcarbamate hemisulfate in 20.0 mL DMF was added 444 mg (2.32 mmol) EDC, 189 mg (1.39 mmol) HOAT, and 426 μL (3.05 mmol) triethylamine. After stirring 6 h at room temperature, the reaction was cooled to 0 °C, and treated dropwise with water to give a precipitate. The solid was removed by filtration, washed well with water, and dried under high vacuum for 24 h to give tert-butyl 2-[({[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetyl}amino) methyl]benzylcarbamate as an off-white solid: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.71-8.65 (m, 2H), 8.82-7.95 (m, 1H), 7.70 (d, 1H, *J* = 7.9Hz), 7.58 (dd, 1H, *J* = 5.3 and 7.4 Hz), 7.41 (t, 1H, *J* = 6.5Hz), 7.34 (br t, 1H, *J* = 5.5Hz), 7.24 (br s, 4H), 6.94 (s, 1H), 4.78 (s, 2H), 4.34 (d, 2H, *J* = 5.7Hz), 4.23 (dt, 2H, *J* = 6.5 and 15.4 Hz), 4.15 (d, 2H, *J* = 5.9Hz), 1.39 (s, 9H); MS (Electrospray): M+Na = 585.2; TLC: R_f = 0.40 (160/10/1 of methylene chloride/methanol/concentrated ammonium hydroxide).

Step B: 1-(2-{[2-(Ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride:

To a suspension of 604 mg (1.07 mmol) tert-butyl 2-[({[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetyl}amino)methyl] benzylcarbamate in 30 mL ethyl acetate at 0 °C was bubbled in hydrogen chloride gas until the solvent was saturated. The reaction was

stirred 20 min in the cold and then concentrated to dryness in vacuo. The solid residue was reconcentrated to dryness in vacuo 2x ethyl acetate and dried under high vacuum for 24 h to give 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.96 (br t, 1H, J = 5.6Hz), 8.70 (d, 1H, J = 4.6Hz), 8.33 (br s, 3H), 8.00 (t, 1H, J = 7.4Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.55 (m, 1H), 7.50-7.41 (m, 2H), 7.37 (br m, 3H), 7.24 (br s, 4H), 6.95 (s, 1H), 4.79 (s, 2H), 4.40 (d, 2H, J = 5.5Hz), 4.23 (dt, 2H, J = 6.3 and 15.3Hz), 4.10 (d, 2H, J = 5.7); MS (Electrospray): M+H = 463.1; TLC: R_f = 0.57 (80/10/1 of methylene chloride/ methanol/concentrated ammonium hydroxide).

EXAMPLE 10

1-(2-{[2-(2-Ammonioethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except tert-butyl 2-[2-(aminomethyl)phenyl]ethylcarbamate is substituted for tert-butyl 2-(aminomethyl)benzylcarbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.94 (br t, 1H, J = 5.4Hz), 8.70 (d, 1H, J = 4.2Hz), 8.09 (br s, 3H), 8.00 (t, 1H, J = 7.7Hz), 7.70 (d, 1H, J = 7.8Hz), 7.60-7.55 (m, 1H), 7.44 (t, 1H, J = 6.5), 7.31-7.20 (br m, 4H), 6.94 (s, 1H), 4.80 (s, 2H), 4.34 (d, 2H, J = 5.5Hz), 4.23 (dt, 2H, J = 6.5 and 15.3Hz), 2.97 (br s, 4H); HRMS (Electrospray): M+H Calcd for $C_{22}H_{24}ClF_2N_6O_2$: 477.1612, Found: 477.1611; TLC: R_f = 0.37 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 11

1-(2-{[2-(2-Ammonioethyl)-5-chlorobenzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except tert-butyl 2-[2-(aminomethyl)-4-chlorophenyl] ethylcarbamate is substituted for tert-butyl 2-(aminomethyl) benzylcarbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.03 (br t, 1H, J = 5.7Hz), 8.70 (d, 1H, J = 4.6Hz), 8.09 (br s, 3H), 8.01 (t, 1H, J = 7.8Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.55 (m, 1H), 7.44 (t, 1H, J = 6.6), 7.33-7.23 (m, 3H), 6.95 (s, 1H), 4.82 (s, 2H), 4.34 (d, 2H, J = 5.7Hz), 4.24 (dt, 2H, J = 6.5 and 15.3Hz), 2.95 (br s, 4H); HRMS (Electrospray): M+H Calcd for $[C_{22}H_{22}Cl_2F_2N_6O_2]H^+$: 511.1222, Found: 511.1224; TLC: R_f = 0.34 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 12

1-(2-{[2-(2-Ammonioethyl)benzyl]amino}-2-oxoethyl)-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and tert-butyl 2-[2-(aminomethyl)phenyl]ethylcarbamate is substituted for tert-butyl 2-(aminomethyl) benzylcarbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.75 (br t, 1H, J = 5.4Hz), 8.70 (d, 1H, J = 4.4Hz), 8.00 (t, 1H, J = 7.8Hz), 7.93 (br s, 3H), 7.72 (d, 1H, J = 7.9Hz), 7.60-7.55 (m, 1H), 7.44 (br s, 1H), 7.34-7.2 (m, 4H), 6.88 (d, 1H, J = 4.6Hz), 6.76 (d, 1H, J = 4.8Hz), 4.84 (s, 2H), 4.37-4.22 (m, 4H), 3.06-2.90 (br m, 4H); HRMS (Electrospray): M+H = 443.1; TLC: R_f = 0.25 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 13

1-(2-{[2-(2-Ammonioethyl)-5-chlorobenzyl]amino}-2-oxoethyl)-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and tert-butyl 2-[2-(aminomethyl)-4-chlorophenyl] ethylcarbamate is substituted for tert-butyl 2-(aminomethyl) benzylcarbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.84 (br t, 1H, J = 5.7Hz), 8.70 (d, 1H, J = 4.3Hz), 8.00 (t, 1H, J = 8.0Hz), 7.95 (br s, 3H), 7.72 (d, 1H, J = 8.1Hz), 7.60-7.55 (m, 1H), 7.43-7.23 (m, 4H), 6.88 (d, 1H, J = 4.5Hz), 6.76 (d, 1H, J = 4.5Hz), 4.59 (s, 2H), 4.36-4.32 (m, 4H), 3.03-2.89 (br m, 4H); HRMS (Electrospray): M+H Calcd for $[C_{22}H_{23}ClF_2N_6O_2]H^+$: 477.1612, Found: 477.1626; TLC: R_f = 0.30 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 14

1-(2-{[2-(Ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxo-1,2-dihydropyrazin-4-ium dichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.94 (br t, 1H, J = 5.5Hz), 8.39-8.30 (m, 4H), 7.62-7.54 (m, 3H), 7.48-7.32 (m, 5H), 6.81 (s, 1H), 4.75 (s, 2H), 4.46 (dt, 2H, J = 6.7 and 13.6), 4.39 (d, 2H, J = 5.5Hz), 4.08 (br d, 2H, J = 5.7); HRMS (Electrospray): M+H = 479.0; TLC: R_f = 0.07 (160/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 15

1-(2-{[2-(2-Ammonioethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxo-1,2-dihydropyrazin-4-ium dichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and tert-butyl 2-[2-(aminomethyl) phenyl]ethylcarbamate is substituted for tert-butyl 2-(aminomethyl) benzylcarbamate hemisulfate in step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.94 (br t, 1H, J = 5.4Hz), 8.36 (d, 1H, J = 6.4Hz), 8.09 (br s, 3H), 7.63-7.52 (m, 3H), 7.40 (t, 1H, J = 7.8Hz), 7.30-7.20 (m, 4H), 6.81 (s, 1H), 4.76 (s, 2H), 4.33 (d, 2H, J = 5.5Hz), 4.46 (2H, obscured), 2.96 (br s, 4H); HRMS (Electrospray): M+H Calcd for $C_{22}H_{24}ClF_2N_6O_3$: 493.1561, Found: 493.1577; TLC: R_f = 0.31 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 16

1-(2-{[2-(2-Ammonioethyl)-5-chlorobenzyl]amino}-2-oxoethyl)-6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxo-1,2-dihydropyrazin-4-ium dichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and tert-butyl 2-[2-(aminomethyl)-4-chlorophenyl] ethylcarbamate is substituted for tert-butyl 2-(aminomethyl)benzyl carbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.98 (br t, 1H, J = 5.8Hz), 8.35 (d, 1H, J = 6.4Hz), 8.04 (br s, 3H), 7.63-7.52 (m, 3H), 7.40 (t, 1H, J = 7.8Hz), 7.33-7.24 (m, 3H), 6.82 (s, 1H), 4.78 (s, 2H), 4.48 (dt, 2H, J = 6.6 and 15.3Hz), 4.33 (d, 2H, J = 5.7Hz), 2.93 (br s, 4H);

HRMS (Electrospray): M+H = 527.2; TLC: R_f = 0.22 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 17

1-(2-{[2-(2-Ammonioethyl)-5-chlorobenzyl]amino}-2-oxoethyl)-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxo-1,2-dihydropyrazin-4-ium dichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid and tert-butyl 2-[2-(aminomethyl)-4-chlorophenyl]ethylcarbamate is substituted for tert-butyl 2-(aminomethyl)benzyl carbamate hemisulfate in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.87 (br t, 1H, J = 5.5Hz), 8.38 (d, 1H, J = 6.3Hz), 8.04 (br s, 3H), 7.81 (br s, 1H), 7.64 (d, 1H, J = 7.9Hz), 7.58 (t, 1H, J = 6.1Hz), 7.42 (t, 1H, J = 8.1Hz), 7.35-7.23 (m, 4H), 6.88 (d, 1H, J = 4.9Hz), 6.76 (d, 1H, J = 4.7Hz), 4.57 (s, 2H), 4.52 (dt, 2H, J = 6.6 and 13.6Hz), 2.96 (br s, 4H); HRMS (Electrospray): M+H Calcd for $C_{22}H_{24}ClF_2N_6O_3$: 493.1561, Found: 493.1562; TLC: R_f = 0.22 (80/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 18

1-(2-{[2-(Ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-phenylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium dichloride:

The title compound is prepared using a similar procedure as described for the synthesis of 1-(2-{[2-(ammoniomethyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium trichloride in EXAMPLE 9 except [6-chloro-3-[(2,2-difluoro-2-phenylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid is substituted for [6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetic acid in Step A: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.00 (br t, 1H, J = 5.5Hz), 8.40(br s, 3H), 7.58-7.43 (m, 7H), 7.37 (br s, 3H), 6.94 (s, 1H), 4.83 (2H, obscured), 4.78 (s, 2H), 4.41 (d, 2H, J = 5.5Hz), 4.13-3.98 (br m, 2H); HRMS (Electrospray): M+H = 462.1; TLC: R_f = 0.19 (160/10/1 of methylene chloride/methanol/ concentrated ammonium hydroxide).

EXAMPLE 19

6-Chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl)amino]methyl}benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate):

To a solution of 50 mg (0.108 mmol) N-[2-(aminomethyl)benzyl]-2-[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetamide in 800 μL methylene chloride was added 15.1 mg (0.124 mmol) DMAP and 9.2 μL methyl chloroformate. The solution was allowed to stand at room temperature for 2 h and purified by reverse phase preparative HPLC (5% to 95% acetonitrile in water containing 0.1% TFA, C18 PRO YMC 20x150 mm) to give 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.72-8.67 (br m, 2H), 7.99 (t, 1H, J = 7.8Hz), 7.70 (d, 1H, J = 7.9Hz), 7.65-7.54 (m, 2H), 7.41 (t, 1H, J = 6.4Hz), 7.25 (br s, 5H), 6.94 (s, 1H), 4.78 (s, 2H), 4.35 (d, 2H, J = 5.5Hz), 4.30-4.18 (m, 4H), 3.55 (s, 3H); MS (Electrospray): M+H = 521.2.

EXAMPLE 20

1-[2-({2-[(Acetylamino)methyl]benzyl}amino)-2-oxoethyl]-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate):

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except acetic anhydride is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73-8.69 (m, 2H), 8.26 (t, 1H, J = 5.5Hz), 7.99 (t, 1H, J = 7.8Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.54 (m, 1H), 7.42 (t, 1H, J = 6.7Hz), 7.25 (s, 4H), 6.94 (s, 1H), 4.78 (s, 2H), 4.34 (d, 2H, J = 5.7Hz), 4.28-4.18 (m, 4H), 1.87 (s, 3H); MS (Electrospray): M+H = 527.0.

EXAMPLE 21

6-Chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methylsulfonyl) amino]methyl}benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate):

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except methanesulfonyl chloride is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73-8.69 (m, 2H), 7.99 (t, 1H, J = 7.8Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.54 (m, 1H), 7.46-7.34 (m, 3H), 7.29 (s, 4H), 6.95 (s, 1H), 4.79 (s, 2H), 4.39 (d, 2H, J = 5.8 Hz), 4.30-4.17 (m, 4H), 1.872.88(s, 3H); MS (Electrospray): M+Na = 563.2.

EXAMPLE 22

6-Chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-(2-{[2-({[(methylamino) carbonyl]amino}methyl)benzyl]amino}-2-oxoethyl)-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate):

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except methyl isocyanate is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.77-8.69 (m, 2H), 8.00 (t, 1H, J = 7.8Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.55 (m, 1H), 7.43 (t, 1H, J = 6.4Hz), 7.28-7.21 (m, 4H), 6.94 (s, 1H), 6.33 (t, 1H, J = 6.0Hz), 5.80 (br s, 1H), 4.78 (s, 2H), 4.34 (d, 2H, J = 5.7Hz), 4.30-4.18 (m, 4H), 2.56 (d, 3H, , J = 4.2Hz); MS (Electrospray): M+Na = 542.1.

EXAMPLE 23

1-(2-{[2-({[(Benzyloxy)carbonyl]amino}methyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate):

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except benzyl chloroformate is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73-8.66 (m, 2H), 7.99 (t, 1H, J = 7.9Hz), 7.77 (t, 1H, J = 5.7Hz), 7.70 (d, 1H, J = 7.9Hz), 7.60-7.54 (m, 1H), 7.44-7.28 (m, 7H), 7.25 (s, 5H), 6.94 (s, 1H), 5.05 (s, 2H), 4.78 (s, 2H), 4.36 (d, 2H, J = 4.8Hz), 4.30-4.18 (m, 4H); MS (Electrospray): M+H = 597.2.

EXAMPLE 24

6-Chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-1-{2-[(2-{[(methoxy carbonyl)amino]methyl}benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium trifluoroacetate:

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except N-[2-(aminomethyl) benzyl]-2-[6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetamide is substituted for N-[2-(aminomethyl)benzyl]-2-[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetamide: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.66 (t, 1H, J = 5.6Hz), 8.35 (d, 1H, J = 6.4Hz), 7.62-7.52 (m, 4H), 7.39 (t, 1H, J = 7.9Hz), 7.23 (s, 4H), 6.80 (s, 1H), 4.74 (s, 2H), 4.46 (dt, 2H, J = 6.4 and 13.3Hz), 4.34 (d, 2H, J = 5.5Hz), 4.20 (d, 2H, J = 5.7Hz), 3.54 (s, 3H); MS (Electrospray): M+H = 537.2.

EXAMPLE 25

6-Chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-1-{2-[(2-{[(methylsulfonyl)amino]methyl}benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium trifluoroacetate:

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except N-[2-(aminomethyl) benzyl]-2-[6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetamide is substituted for N-[2-(aminomethyl)benzyl]-2-[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetamide and methanesulfonyl chloride is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.69 (t, 1H, J = 5.5 Hz), 8.35 (d, 1H, J = 6.4Hz), 7.62-7.52 (m, 3H), 7.45-7.34 (m, 3H, J = 7.9Hz), 7.30-7.25 (m, 3H), 6.80 (s, 1H), 4.75 (s, 2H), 4.46 (dt, 2H, J = 6.2 and 13.4Hz), 4.37 (d, 2H, J = 6.0Hz), 4.18 (d, 2H, J = 56.0Hz), 2.88 (s, 3H); MS (Electrospray): M+H = 557.2.

EXAMPLE 26

1-(2-{[2-({[(Benzyloxy)carbonyl]amino}methyl)benzyl]amino}-2-oxoethyl)-6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxo-1,2-dihydropyrazin-4-ium trifluoroacetate:

The title compound is prepared using a similar procedure as described for the synthesis of 6-chloro-3-[(2,2-difluoro-2-pyridinium-2-ylethyl)amino]-1-{2-[(2-{[(methoxycarbonyl) amino]methyl} benzyl)amino]-2-oxoethyl}-2-oxo-1,2-dihydropyrazin-4-ium bis(trifluoroacetate) in EXAMPLE 19 except N-[2-(aminomethyl) benzyl]-2-[6-chloro-3-{[2,2-difluoro-2-(1-oxidopyridin-2-yl)ethyl]amino}-2-oxopyrazin-1(2H)-yl]acetamide is substituted for N-[2-(aminomethyl)benzyl]-2-[6-chloro-3-[(2,2-difluoro-2-pyridin-2-ylethyl)amino]-2-oxopyrazin-1(2H)-yl]acetamide and benzyl chloroformate is substituted for methyl chloroformate: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.67 (t, 1H, J = 5.2Hz), 8.35 (d, 1H, J = 6.4Hz), 7.77 (t, 1H, J = 5.8Hz), 7.63-7.52 (m, 3H), 7.41-7.28 (m, 6H), 7.24 (s, 5H), 6.80 (s, 1H), 5.04 (s, 2H), 4.74 (s, 2H), 4.46 (dt, 2H, J = 6.7 and 13.5Hz), 4.35 (d, 2H, J = 5.3Hz), 4.23 (m, 2H, J = 5.7Hz); MS (Electrospray): M+H = 613.3.

EXAMPLE 27

N-[2-(aminomethyl)-5-chlorobenzyl]-2-(6-methyl-2-oxo-3-[(2-phenylethyl)amino]-1(2H)-pyrazinyl)acetamide

STEP A. *tert*-butyl 4-chloro-2-({[(6-methyl-2-oxo-3-[(2-phenylethyl)amino]-1(2*H*)-pyrazinyl)acetyl]amino}methyl)benzylcarbamate

To a stirred solution of *tert*-butyl 2-(aminomethyl)-4-chlorobenzylcarbamate and [6-methyl-2-oxo-3-[(2-phenylethyl)amino]pyrazin-1(2*H*)-yl]acetic acid (J. Med. Chem. 1998, 41, 4466) in DMF (0.3 ml) was added EDC (10.1 mg, 0.3 mmol) and HOAT (4.8 mg, 0.03 mmol). The mixture was stirred overnight at room temperature. Water (3 ml) was added to the crude reaction causing a heavy white precipitate to form, which was filtered yielding a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.21- 7.52 (m, 8 H); 6.76 (s, 1 H); 4.62 (s, 2 H); 4.42 (d, 2 H, J= 5.86 Hz); 4.25 (d, 2 H, 6.05 Hz); 3.65 (dd, 2 H, J= 13.37,6.96 Hz); 2.91 (m, 2 H); 2.01 (S, 3 H); 1.57 (s, 9 H).

STEP B. *N*-[2-(aminomethyl)-5-chlorobenzyl]-2-(6-methyl-2-oxo-3-[(2-phenylethyl)amino]-1(2*H*)-pyrazinyl)acetamide

Through a stirred solution of *tert*-butyl 4-chloro-2-({[(6-methyl-2-oxo-3-[(2-phenylethyl)amino]-1(2*H*)-pyrazinyl)acetyl]amino}methyl)benzylcarbamate in dichloromethane (5 ml) at 0°C was bubbled HCl gas for 5 minutes. After stirring at room temperature for one half hour, Ar was bubbled through the suspension for several minutes. The solvent was concentrated in vacuo and the product was dried under vacuum overnight, yielding the dihydrochloride salt of *N*-[2-(aminomethyl)-5-chlorobenzyl]-2-(6-methyl-2-oxo-3-[(2-phenylethyl)amino]-1(2*H*)-pyrazinyl)acetamide.

^{1}H NMR (CD3OD, 400 MHz): δ 7.23-7.47 (m, 8 H); 6.55 (s 1 H); 4.85 (b s, 2 H); 4.48 (b s, 2 H); 3.68 (m, 2 H); 3.0 (m, 2 H); 2.17 (s, 3 H).

EXAMPLE 28

N-[2-(aminomethyl)-5-chlorobenzyl]-2-(3-[(benzylsulfonyl)amino]-6-methyl-2-oxo-1(2*H*)-pyridinyl)acetamide

Prepared by a procedure similar to that described in Example 27 except substituting [3-[(benzylsulfonyl)amino]-6-methyl-2-oxopyridin-1(2*H*)-yl]acetic acid (J. Med. Chem. 1998, 41, 4466)in Step A.

H NMR (CD_3OD, 400 MHz): δ 7.51 (b s, 1 H); 7.41 (m, 2 H), 7.26-7.40 (m, 6 H); 6.14 (d, 1 H, 7.6 Hz); 4.85 (s, 2 H); 4.49 (s, 2 H); 4.32 (s, 2 H); 4.24 (s, 2 H); 2.29 (s, 3 H).

EXAMPLE 29

N-[2-(aminomethyl)-5-chlorobenzyl]-2-(3-{[2,2-difluoro-2-(1-oxido-2-pyridinyl)ethyl]amino}-2-oxo-1(2*H*)-pyrazinyl)acetamide

H NMR (CD_3OD, 400 MHz): δ 850 (b s, 1 H); 7.86-7.88 (m , 1 H), 7.70-7.72 (m, 2 H); 7.38-7.47 (m, 3 H); 6.98-7.00 (m, 1 H); 6.80-6.82 (m 1 H); 4.65-4.68 (m, 2 H); 4.48 (b s, 2 H); 4.25 (b s, 2 H)

EXAMPLE 30

N-[2-(aminomethyl)-5-chlorobenzyl]-2-(6-chloro-3-{[2,2-difluoro-2-(1-oxido-2-pyridinyl)ethyl]amino}-2-oxo-1(2*H*)-pyrazinyl)acetamide

H NMR (CD_3OD, 400 MHz): δ 8.49 (b s, 1 H);); 7.83-7.86 (m, 1 H), 7.69-7.71 (m, 2 H); 7.38-7.46 (m, 3 H); 6.90 (s, 1 H); 4.89 (s, 2 H); 4.62 (t, 2 H, J = 13.73 Hz); 4.47 (s, 2 H); 4.25 (s, 2 H).

EXAMPLES 31A and 31B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-n-propylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-n-propylaminomethyl-benzyl)-acetamide

Step A. 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-n-propylaminomethyl-benzyl)-acetamide

To a solution of 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-aminomethyl-benzyl)-acetamide (10 mg, 0.022 mmol) in DCE (0.4 ml) is added propionaldehyde (100 ul of a solution of 121 ul propionaldehyde in 5 ml DCE, 0.04 mmol) and $NaBH(OAc)_3$ (8.2 mg, 0.04 mmol). The resulting mixture is sonicated for 30 sec and allowed to stand at room temperature for 18 h. The solvent is blown down with nitrogen and the crude material is dissolved in DMF (400 ul) and water (40 ul), and purified by reverse phase preparative HPLC (5% to 95% CH_3CN in water containing 0.1 % TFA, C18 PRO YMC 20x150 mm) to provide after solvent evaporation: 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-n-propylaminomethyl-benzyl)-acetamide: ^{1}H NMR (CD_3OD, 400 MHz): δ 9.3 (br t, 0.5 H); 8.62 (d, J = 5.5 Hz, 1 H); 7.95 (dt, J = 6.5, 1.3 Hz, 1 H); 7.72 (d, J = 6.5 Hz, 1 H); 7.55-7.35 (m, 5 H); 6.85 (s, 1 H); 4.85 (s, 2 H); 4.48 (s, 2 H); 4.33 (s, 2 H); 4.28 (t, J = 14 Hz, 2 H); 3.02 (t, J = 7.5 Hz, 2 H);1.75-1.6 (m, 2 H); 0.95 (t, J = 7.5 Hz, 3 H);. MS (ES) M+1 505.61 for $C_{24}H_{27}ClF_2N_6O_2$

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-n-propylaminomethyl-benzyl)-acetamide: ^{1}H NMR (CD_3OD, 400 MHz): δ 9.58 (br t, 0.5 H); 8.63 (d, J = 5.5 Hz, 1 H); 7.95 (dt, J = 6.5, 1.3 Hz, 1 H); 7.71 (d, J = 6.5 Hz, 1 H); 7.57-7.35 (m, 5 H); 6.83 (s, 1 H); 4.85 (s, 2 H); 4.51 (s, 2 H); 4.42 (br s, 2 H); 4.28 (t, J = 14 Hz, 2 H); 3.18-2.96 (m, 4 H); 1.87-1.52 (m, 4 H); 0.9 (t, J = 7.5 Hz, 3 H). MS (ES) M+1 547.65 for $C_{27}H_{33}ClF_2N_6O_2$

EXAMPLE 32A

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-methylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-methylaminomethyl-benzyl)-acetamide, MS (ES) M+1 477.5 for $C_{22}H_{23}ClF_2N_6O_2$, is prepared by coupling the acid of Example 5 and N-(t-Butoxycarbonyl)-N-methyl-(2-aminomethyl)-benzylamine,

followed by Boc removal, using a similar procedure as described in Step b of Example 9. N-(t-Butoxycarbonyl)-N-methyl-(2-aminomethyl)-benzylamine is prepared as follows: N-(t-Butoxycarbonyl)- 2-aminomethyl)-benzylamine sulfate (200 mg, 0.702 mmol) was partitioned between $CHCl_3$ and aqueous sodium bicarbonate. The combined organic layer was dried on sodium sulfate, concentrated in vacuo and azeotroped with benzene. To a solution of the resulting N-(t-Butoxycarbonyl)- 2-aminomethyl)-benzylamine free base in DMF(5 ml) cooled to 0 °C, was added sodium bis(trimethylsilyl)amide (0.7 ml of 1M solution in THF, 0.7 mmol). The solution was stirred at 0 °C for 15 min and dimethyl sulfate (63 ul, 0.67 mmol) was added slowly. The reaction mixture was stirred at 0 °C for 80 min and allowed to warm to room temperature. The reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was washed with aqueous LiCl, dried over sodium sulfate and concentrated in vacuo. Purification by flash chromatography (silica gel, 2% MeOH containing 10% NH_4OH in CH_2Cl_2 to 8%) provided N-(t-Butoxycarbonyl)-N-methyl-(2-aminomethyl)-benzylamine: ^1H NMR (CD_3OD, 400 MHz): δ 7.36 (br d, J = 7.5 Hz, 1 H); 7.32-7.2 (m, 2 H); 7.16 (br d, J = 7.5 Hz, 1 H); 4.55 (s, 2 H); 3.9 (s, 2 H); 2.8 (s, 3 H); 1.45 (s, 9 H).

EXAMPLE 32b

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-dimethylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-dimethylaminomethyl-benzyl)-acetamide is prepared from formaldehyde using a procedure similar as the one described in example 31: MS (ES) M+1 491.5 for $C_{23}H_{25}ClF_2N_6O_2$

EXAMPLE 33A and 33B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-ethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-diethylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-ethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-diethylaminomethyl-benzyl)-acetamide were prepared from acetaldehyde using a procedure similar as the one described in example 31: MS (ES) M+1 491.4 for $C_{23}H_{25}ClF_2N_6O_2$ and MS (ES) M+1 519.5 for $C_{25}H_{29}ClF_2N_6O_2$.

EXAMPLE 34A and 34B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-n-butylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-n-buthylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-n-butylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-n-buthylaminomethyl-benzyl)-acetamide were prepared from butyraldehyde using a procedure similar as the one described in example 1: MS (ES) M+1 519.48 for $C_{25}H_{29}ClF_2N_6O_2$ and MS (ES) M+1 575.5 for $C_{29}H_{37}ClF_2N_6O_2$.

EXAMPLE 35A and 35B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-isobutylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-isobutylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-isobutylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-isobuthylaminomethyl-benzyl)-acetamide were prepared from isobutyraldehyde using a procedure similar as the one described in example 31: MS (ES) M+1 519.4 for $C_{25}H_{29}ClF_2N_6O_2$ and MS (ES) M+1 575.5 for $C_{29}H_{37}ClF_2N_6O_2$.

EXAMPLE 36A and 36B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopropylmethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-cyclopropylmethylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopropylmethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-cyclopropylmethylaminomethyl-benzyl)-acetamide were prepared from cyclopropyl aldehyde using a procedure similar as the one described in example 31: MS (ES) M+1 517.4 for $C_{25}H_{27}ClF_2N_6O_2$ and MS (ES) M+1 571.5 for $C_{29}H_{33}ClF_2N_6O_2$.

EXAMPLE 37

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-benzylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-benzylaminomethyl-benzyl)-acetamide was prepared from phenylacetaldehyde using a procedure similar as the one described in example 1: MS (ES) M+1 553.4 for $C_{28}H_{27}ClF_2N_6O_2$

EXAMPLE 38A and 38B

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-phenethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-phenethylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-phenethylaminomethyl-benzyl)-acetamide and 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-bis-phenethylaminomethyl-benzyl)-acetamide were prepared from phenylacetalaldehyde using a procedure similar as the one described in example 31: MS (ES) M+1 567.4 for $C_{29}H_{29}ClF_2N_6O_2$ and MS (ES) M+1 671.5 for $C_{37}H_{37}ClF_2N_6O_2$.

EXAMPLE 39

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-isopropylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-isopropylaminomethyl-benzyl)-acetamide was prepared from acetone using a procedure similar as the one described in example 1: MS (ES) M+1 505.5 for $C_{24}H_{27}ClF_2N_6O_2$

EXAMPLE 40

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclobutylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclobutylaminomethyl-benzyl)-acetamide was prepared from cyclobutanone using a procedure similar as the one described in example 1: MS (ES) M+1 517.4 for $C_{25}H_{27}ClF_2N_6O_2$

EXAMPLE 41

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopentylaminomethyl-benzyl)-acetamide

2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopentylaminomethyl-benzyl)-acetamide was prepared from cyclobutanone using a procedure similar as the one described in example 31: MS (ES) M+1 531.4 for $C_{26}H_{29}ClF_2N_6O_2$

Typical tablet cores suitable for administration of thrombin inhibitors are comprised of, but not limited to, the following amounts of standard ingredients:

Excipient	General Range (%)	Preferred Range (%)	Most Preferred Range (%)
mannitol	10-90	25-75	30-60
microcrystalline cellulose	10-90	25-75	30-60
magnesium stearate	0.1-5.0	0.1-2.5	0.5-1.5

Mannitol, microcrystalline cellulose and magnesium stearate may be substituted with alternative pharmaceutically acceptable excipients.

The thrombin inhibitors can also be co-administered with suitable anti-platelet agents, including, but not limited to, fibrinogen receptor antagonists (e.g. to treat or prevent unstable angina or to prevent reocclusion after angioplasty and restenosis), anticoagulants such as aspirin, thrombolytic agents such as plasminogen activators or streptokinase to achieve synergistic effects in the treatment of various vascular pathologies, or lipid lowering agents including antihypercholesterolemics (e.g. HMG CoA reductase inhibitors such as lovastatin, HMG CoA synthase inhibitors, etc.) to treat or prevent atherosclerosis. For example, patients suffering from coronary artery disease, and patients subjected to angioplasty procedures, would benefit from coadministration of fibrinogen receptor antagonists and thrombin inhibitors. Also, thrombin inhibitors enhance the efficiency of tissue plasminogen activator-mediated thrombolytic reperfusion. Thrombin inhibitors may be administered first following thrombus formation, and tissue plasminogen activator or other plasminogen activator is administered thereafter.

Typical doses of thrombin inhibitors of the invention in combination with other suitable anti-platelet agents, anticoagulation agents, or thrombolytic agents may be the same as those doses of thrombin inhibitors administered without coadministration of additional anti-platelet agents, anticoagulation agents, or thrombolytic agents, or may be substantially less that those doses of thrombin inhibitors administered without coadministration of additional anti-platelet agents, anticoagulation agents, or thrombolytic agents, depending on a patient's therapeutic needs.

In Vitro Assay For Determining Proteinase Inhibition

Assays of human α-thrombin and human trypsin were performed by the methods substantially as described in Thrombosis Research, Issue No. 70, page 173 (1993) by S.D. Lewis et al.

The assays were carried out at 25°C in 0.05 M TRIS buffer pH 7.4, 0.15 M NaCl, 0.1% PEG. Trypsin assays also contained 1 mM $CaCl_2$. In assays wherein rates of hydrolysis of a p-nitroanilide (pna) substrate were determined, a Thermomax 96-well plate reader was used was used to measure (at 405 nm) the time dependent appearance of p-nitroaniline. sar-PR-pna was used to assay human α-thrombin (K_m=125 μM) and bovine trypsin (K_m=125 μM). p-Nitroanilide substrate concentration was determined from measurements of absorbance at 342 nm using an extinction coefficient of 8270 $cm^{-1}M^{-1}$.

In certain studies with potent inhibitors (K_i < 10 nM) where the degree of inhibition of thrombin was high, a more sensitive activity assay was employed. In this assay the rate of thrombin catalyzed hydrolysis of the fluorogenic substrate Z-GPR-afc (K_m=27 μM) was determined from the increase in fluorescence at 500 nm (excitation at 400 nm) associated with production of 7-amino-4-trifluoromethyl coumarin. Concentrations of stock solutions of Z-GPR-afc were determined from measurements of absorbance at 380 nm of the 7-amino-4-trifluoromethyl coumarin produced upon complete hydrolysis of an aliquot of the stock solution by thrombin.

Activity assays were performed by diluting a stock solution of substrate at least tenfold to a final concentration $\leq 0.1\ K_m$ into a solution containing enzyme or enzyme equilibrated with inhibitor. Times required to achieve equilibration between enzyme and inhibitor were determined in control experiments. Initial velocities of product formation in the absence (V_o) or presence of inhibitor (V_i) were measured. Assuming competitive inhibition, and that unity is negligible compared $K_m/[S]$, [I]/e, and [I]/e (where [S], [I], and e respectively represent the total concentrations, of substrate, inhibitor and enzyme), the equilibrium constant (K_i) for dissociation of the inhibitor from the enzyme can be obtained from the dependence of V_o/V_i on [I] shown in the following equation.

$$V_o/V_i = 1 + [I]/K_i$$

The activities shown by this assay indicate that the compounds of the invention are therapeutically useful for treating various conditions in patients suffering from unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, and reocclusion or restenosis of recanalized vessels.

EXAMPLE 42

Tablet Preparation

Tablets containing 25.0, 50.0, and 100.0 mg., respectively, of the following active compounds are prepared as illustrated below (compositions A-C). Active I is compound 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopentylaminomethyl-benzyl)-acetamide.

	Amount-(mg)		
Component	A	B	C
Active I	25	50	100
Microcrystalline cellulose	37.25	100	200
Modified food corn starch	37.25	4.25	8.5
Magnesium stearate	0.5	0.75	1.5

All of the active compound, cellulose, and a portion of the corn starch are mixed and granulated to 10% corn starch paste. The resulting granulation is sieved, dried and blended with the remainder of the corn starch and the magnesium stearate. The resulting granulation is then compressed into tablets containing 25.0, 50.0, and 100.0 mg, respectively, of active ingredient per tablet.

EXAMPLE 43

Tablet Preparation

Exemplary compositions of compound 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopentylaminomethyl-benzyl)-acetamide (Active I) tablets are shown below:

Component	0.25 mg	2 mg	10 mg	50 mg
Active I	0.500%	1.000%	5.000%	14.29%
mannitol	49.50%	49.25%	47.25%	42.61%
microcrystalline cellulose	49.50%	49.25%	47.25%	42.61%
magnesium stearate	0.500%	0.500%	0.500%	0.500%

2, 10 and 50 mg tablets were film-coated with an aqueous dispersion of hydroxypropyl cellulose, hydroxypropyl methylcellulose and titanium dioxide, providing a nominal weight gain of 2.4%.

Tablet preparation via direct compression

Active I, mannitol and microcrystalline cellulose were sieved through mesh screens of specified size (generally 250 to 750 μm) and combined in a suitable blender. The mixture was subsequently blended (typically 15 to 30 min) until the drug was uniformly distributed in the resulting dry powder blend. Magnesium stearate was screened and added to the blender, after which a precompression tablet blend was achieved upon additional mixing (typically 2 to 10 min). The precompression tablet blend was then compacted under an applied force, typically ranging from 0.5 to 2.5 metric tons, sufficient to yield tablets of suitable physical strength with acceptable disintegration times (specifications will vary with the size and potency of the compressed tablet). In the case of the 2, 10 and 50 mg potencies, the tablets were dedusted and film-coated with an aqueous dispersion of water-soluble polymers and pigment.

Tablet preparation via dry granulation

Alternatively, a dry powder blend is compacted under modest forces and remilled to afford granules of specified particle size. The granules are then mixed with magnesium stearate and tabletted as stated above.

EXAMPLE 44

Intravenous Formulations

Intravenous formulations of compound 2-[6-chloro-3-(2,2-difluoro-2-pyridin-2-yl-ethylamino)-2-oxo-2H-pyrazin-1-yl]-N-(2-cyclopentylaminomethyl-benzyl)-acetamide (Active I) were prepared according to general intravenous formulation procedures.

Component	Estimated range
Active I	0.12 - 0.61 mg
D-glucuronic acid*	0.5 - 5 mg
Mannitol NF	50-53 mg
1 N Sodium Hydroxide	q.s. pH 3.9 - 4.1
Water for injection	q.s. 1.0 mL

Exemplary compositions A-C are as follows:

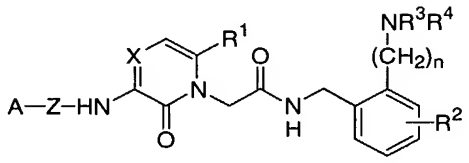
Component	A	B	C
Active I	0.61 mg*	0.30**	0.15***
D-glucuronic acid*	1.94 mg	1.94 mg	1.94 mg
Mannitol NF	51.2 mg	51.2 mg	51.2 mg
1 N Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for injection	q.s. 1.0 mL	q.s. 1.0 mL	q.s. 1.0 mL

* 0.50 mg free base ** 0.25 mg free base *** 0.12 mg free base

Various other buffer acids, such as L-lactic acid, acetic acid, citric acid or any pharmaceutically acceptable acid/conjugate base with reasonable buffering capacity in the pH range acceptable for intravenous administration may be substituted for glucuronic acid.

WHAT IS CLAIMED IS:

1. A compound of the general formula:



and pharmaceutically acceptable salts thereof, wherein

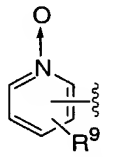
n is 1 or 2;

A is

1) a 6-membered non-heterocyclic unsaturated ring system, unsubstituted, monosubstituted, disubstituted, or trisubstituted, same or different, with C_{1-4} alkyl;

2) a 6-membered heterocyclic unsaturated or saturated ring system wherein 1 ring atom is selected from the group of heteroatoms consisting of N, O and S, wherein the ring carbons are unsubstituted, monosubstituted, disubstituted, or trisubstituted, same or different, with C_{1-4} alkyl, or

3)



, where R^9 is hydrogen or C_{1-8}alkyl;

Z is $-(CH_2)_{2-4}-$, $-CF_2(CH_2)_{1-3}-$, or $-(CH_2)_{1-3}SO_2-$;

X is CH or N;

R^1 is halogen;

R^2 is hydrogen or halogen; and

R^3 and R^4 are independently selected from the group consisting of

hydrogen,

$-C(O)R^5$, where R^5 is selected from the group consisting of $OC(CH_3)$, OCH_3, CH_3, $NHCH_3$, and OCH_2R^6, where R^6 is phenyl;

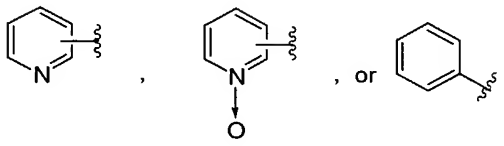
C_{1-4} alkyl,

C_{3-7} cycloalkyl,

$-(CH_2)_{1-2}R^7$, where R^7 is selected from the group consisting of C_{3-7} cycloalkyl and phenyl,

$-SO_2R^8$, where R^8 is C_{1-4}alkyl.

2. A compound of Claim 1, or pharmaceutically acceptable salt thereof, wherein R^1 is Cl, and A is



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3. A compound of Claim 2, or pharmaceutically acceptable salt thereof, R^3 and R^4 are independently selected from the group consisting of

hydrogen,

$-C(O)R^5$, where R^5 is selected from the group consisting of $OC(CH_3)$, OCH_3, CH_3, $NHCH_3$, and OCH_2R^6, where R^6 is phenyl;

C_{1-4} alkyl,

cyclobutyl,

cyclopentyl,

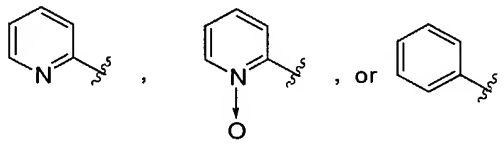
$-(CH_2)_{1-2}R^7$, where R^7 is selected from the group consisting of cyclopropyl and phenyl, and

$-SO_2CH_3$.

4. A compound of Claim 3, or pharmaceutically acceptable salt thereof, wherein

X is CH or N; Z is $-CH_2CH_2-$, $-CF_2CH_2-$, -CH2SO2-;

A is



;

R^1 is Cl;

R^2 is hydrogen or Cl;

R^3 and R^4 are independently selected from the group consisting of

hydrogen,

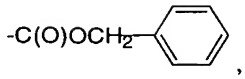
$-C(O)OC(CH_3)_3$,

$-C(O)OCH_3$,

$-C(O)CH_3$

$-SO_2CH_3$,

$-C(O)NHCH_3$


,

$-CH_2CH_2CH_3$,

$-CH_3$,

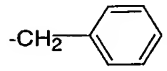
$-CH_2CH_3$,

$-CH_2CH_2CH_2CH_3$,

$-CH_2CH(CH_3)_2$,

$-C(CH_3)_3$,


,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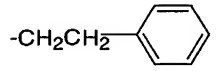
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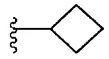
$-CH(CH_3)_2$,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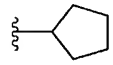
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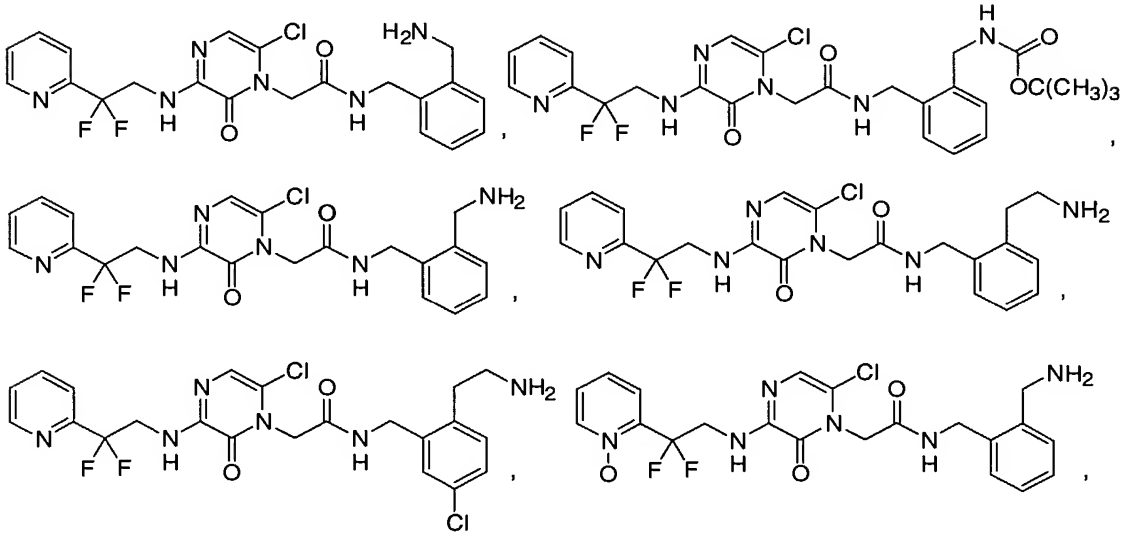
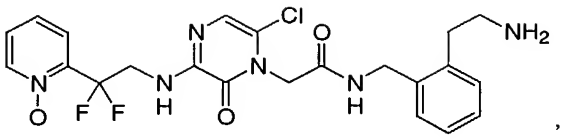
5. A compound of Claim 4, or pharmaceutically acceptable salt thereof, selected from the group consisting of

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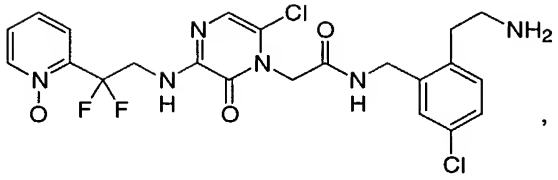
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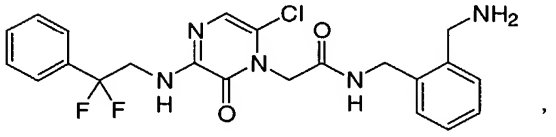
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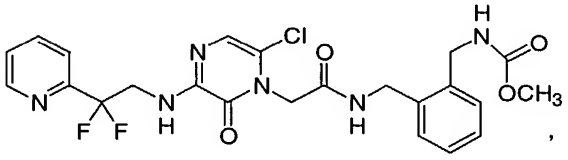
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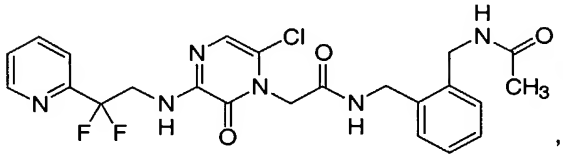
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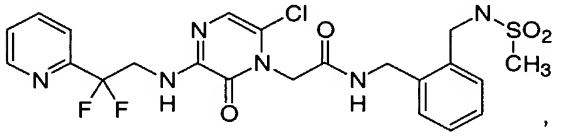
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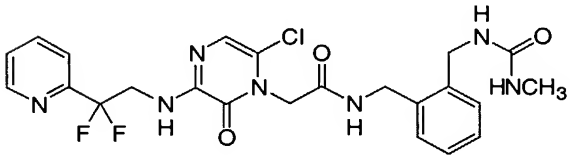
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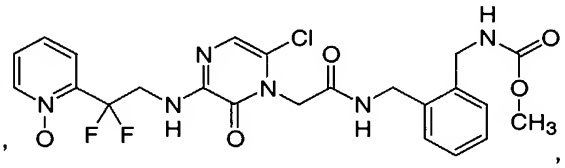
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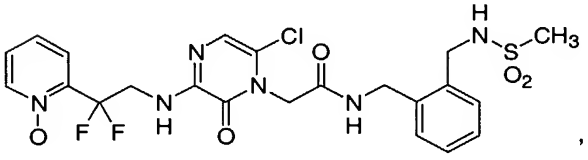
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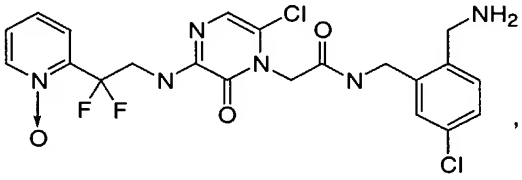
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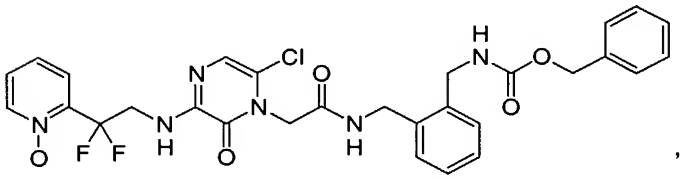
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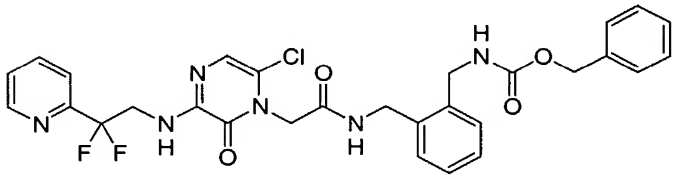
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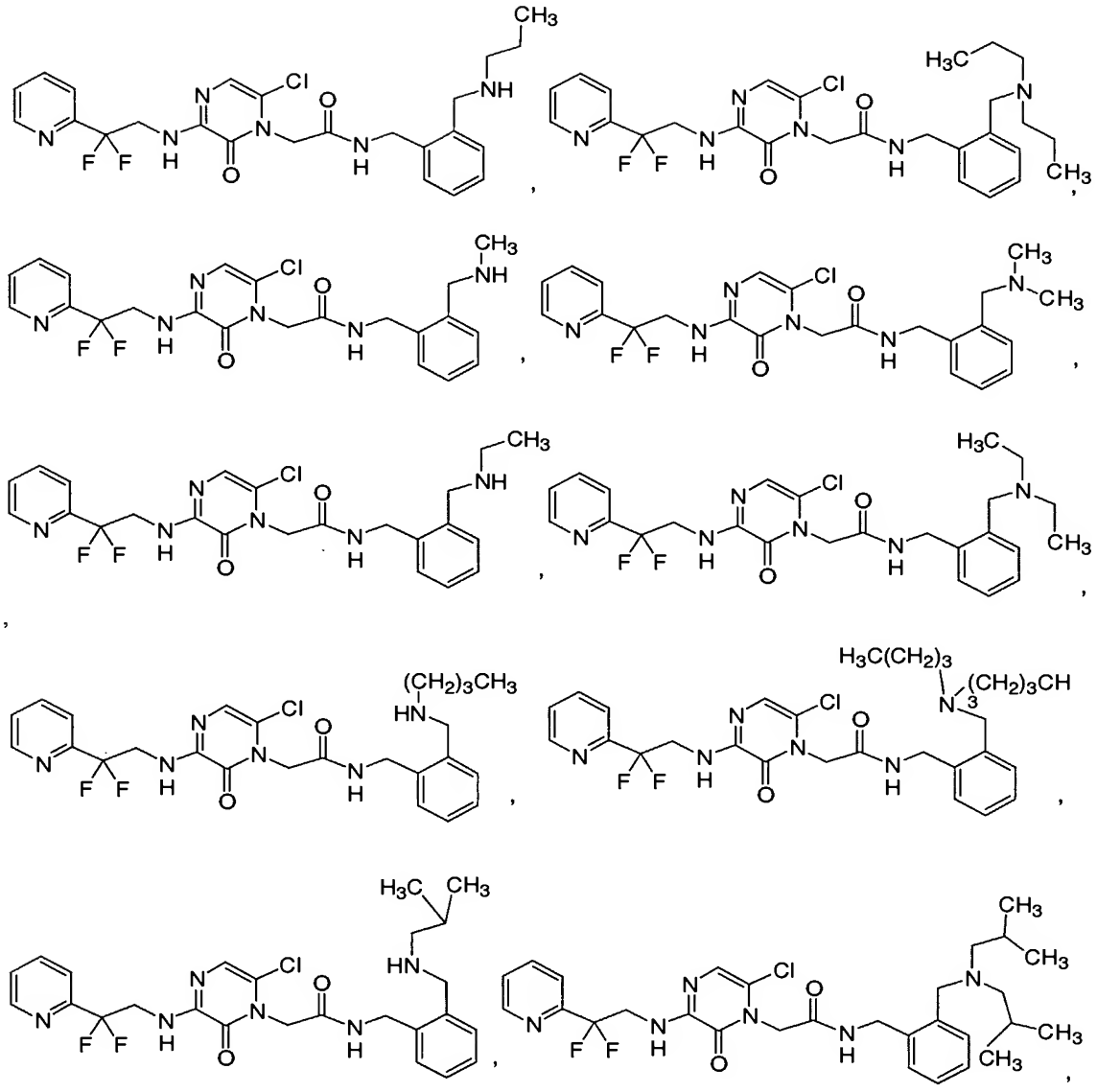

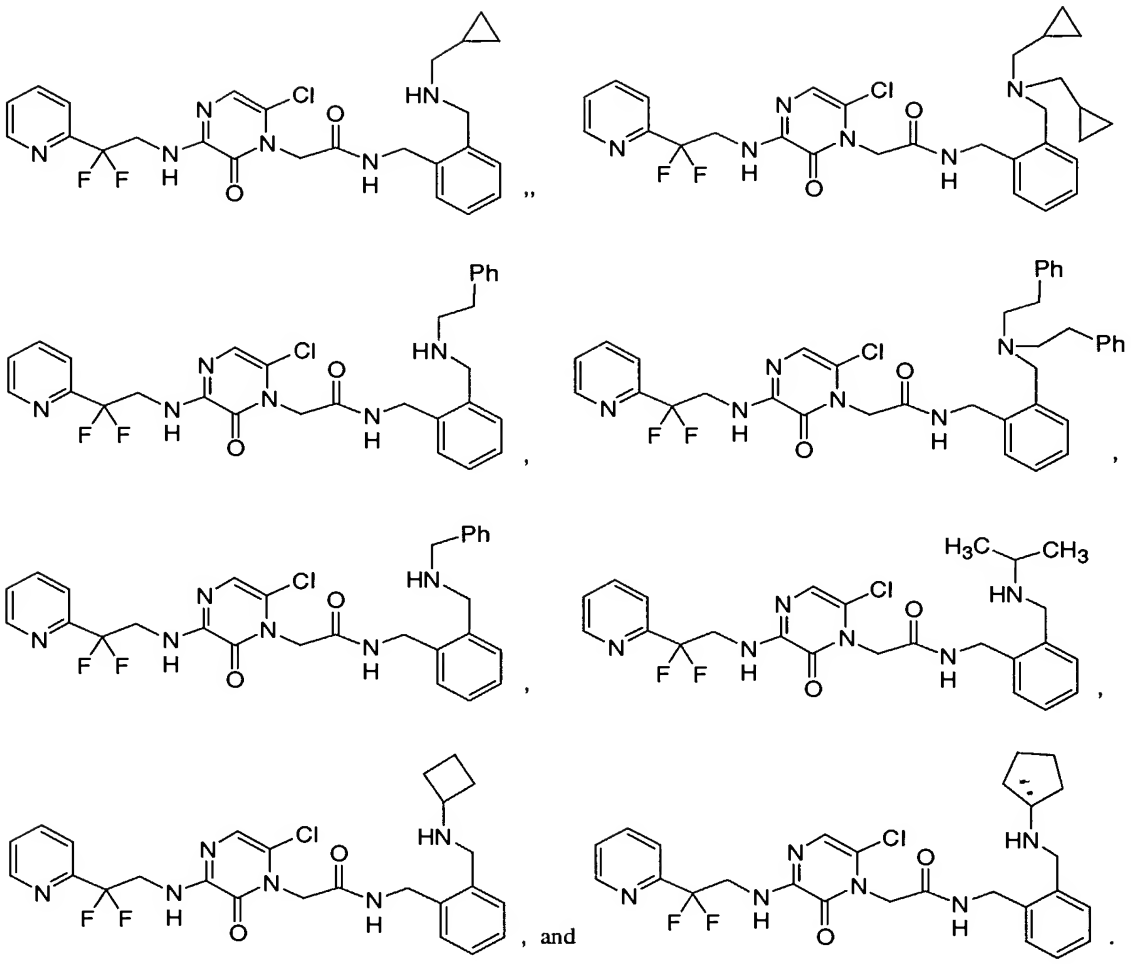

6. A composition for inhibiting thrombus formation in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

7. A method for inhibiting thrombin in blood comprising adding to the blood a composition of Claim 6.

8. A method for inhibiting formation of blood platelet aggregates in blood comprising adding to the blood a composition of Claim 6.

9. A method for inhibiting thrombus formation in blood comprising adding to the blood a composition of Claim 6.

10. The use of a compound of Claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inhibiting thrombin, inhibiting thrombus formation, treating thrombus formation, or preventing thrombus formation in a mammal.

11. A method for treating or preventing venous thromboembolism and pulmonary embolism in a mammal comprising administering to the mammal a composition of Claim 6.

12. A method for treating or preventing deep vein thrombosis in a mammal comprising administering to the mammal a composition of Claim 6.

13. A method for treating or preventing thromboembolic stroke in humans and other mammals comprising administering to the mammal a composition of Claim 6.